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APPROVAL SHEET

Title of Dissertation: "Effects of nicotine and ethanol on indices of reward and sensory-motor function in rats"

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Doctor of Philosophy Degree
7 October 1997

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Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE **1997**	2. REPORT TYPE	3. DATES COVERED **00-00-1997 to 00-00-1997**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Effects of Nicotine and Ethanol on Indices of Reward and Sensory-Motor Function in Rats: Implications for the Positive Epidemiologic Relationship Between the Use of Cigarettes and the Use of Alcohol	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Uniformed Services University of the Health Sciences,F. Edward Hebert School of Medicine,4301 Jones Bridge Road,Bethesda,MD,20814-4799**	8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
see report

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES **170**	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

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ABSTRACT

Title of Thesis: "Effects of Nicotine and Ethanol on Indices of Reward and Sensory-Motor Function in Rats: Implications for the Positive Epidemiologic Relationship Between the Use of Cigarettes and the use of Alcohol"

Eric Jon Popke, Doctor of Philosophy, 1997

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The present experiments examined effects of nicotine and ethanol on behavioral and biochemical indices of reward and sensory-motor function in rats. In Experiment 1, the conditioned place preference paradigm was used to assess nicotine reward following acute treatment with ethanol or saline. Contrary to predictions, there was no evidence of place conditioning by nicotine and no effects of ethanol to alter nicotine place preference. There was, however, an effect of nicotine to offset the locomotor depressant effects of ethanol as indexed by the number of crosses between two shuttle-box chambers. In addition, there was a significant effect of nicotine and ethanol to reduce the ratio of dopamine/DOPAC in nucleus accumbens. Because dopaminergic activity in nucleus accumbens is known to mediate nicotine reinforcement, reductions in the ratio of dopamine/DOPAC (perhaps indicating an increase in the rate of dopamine turnover) suggest a biologic mechanism that may motivate some smokers to smoke more when they drink.

In Experiment 2, the acoustic startle response paradigm was used to assess effects of nicotine and ethanol on behavioral indices of sensory-motor

function in rats. Results indicate a significant interaction of nicotine with ethanol to influence the acoustic startle response (ASR) and to influence pre-pulse inhibition of the acoustic startle response (PPI). More specifically, nicotine administration attenuated effects of low-dose ethanol to increase ASR and PPI. These data were interpreted as evidence that nicotine and ethanol can interact to influence sensory-motor function. Because some smokers may smoke to regulate psychomotor function, these results suggest a behavioral mechanism that may motivate some individuals to smoke more when they drink. Biochemical analyses revealed an effect of ethanol to attenuate nicotine-induced depletions of DOPAC in striatum and an interaction of ethanol with nicotine to alter the ratios of dopamine/l-DOPA, dopamine/DOPAC, and dopamine/HVA in striatum. Because dopaminergic activity in striatum is known to modulate ASR and PPI, these results suggest a mechanism by which nicotine and ethanol may interact to alter sensory-motor function. Implications of these experiments are discussed as they may relate to the positive epidemiologic relationship between the use of cigarettes and the use of alcohol.

Effects of Nicotine and Ethanol on Indices of Reward and Sensory-

Motor Function in Rats: Implications for the Positive

Epidemiologic Relationship Between the Use of

Cigarettes and the use of Alcohol"

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Dissertation Submitted to the Faculty of the
Department of Medical and Clinical Psychology
Graduate Program of the Uniformed Services University
of the Health Sciences in Partial Fulfillment
of the Requirements for the degree of
Doctor of Philosophy
1997

ACKNOWLEDGMENTS

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INTRODUCTION

Cigarette smoking is the single largest cause of preventable death in the United States today, accounting for more than 500,000 premature deaths each year (Peto, Lopez, Boreham, Thun, & Heath, 1994). Because cigarette smoking presents such an important public health problem, it is essential to understand situational variables that lead to increased smoking. One such variable is alcohol consumption. Available evidence indicates that a positive epidemiologic relationship exists between cigarette smoking and alcohol consumption (Friedman, Sieglaub, & Seltzer, 1974; Kaprio Hammar, Koskenvuo, Floderus-Myrhed, Langvanio, et al., 1982; Kozlowski, Henningfield, Keenan, Lei, Leigh, et al., 1993). Additional evidence implicates alcohol as a risk factor for relapse during smoking cessation (Borland, 1990). Yet, the mechanisms that underlie this relationship have not been established. The purpose of the present experiments was to examine behavioral and biological effects of nicotine (the primary addictive constituent of tobacco smoke [USDHHS, 1988]) and ethanol (the most widely abused form of alcohol) in rats that may help to explain why many people smoke and drink concurrently.

In Experiment 1, effects of nicotine to induce conditioned place preference was examined as a behavioral index of nicotine reward. Ethanol then was administered prior to place-preference testing to examine the reward efficacy of nicotine in the presence or absence of ethanol. Results indicating that ethanol can potentiate nicotine-induced place preference would suggest that ethanol can enhance nicotine reward. Results indicating that ethanol can

attenuate nicotine-induced place preference would suggest that ethanol can attenuate nicotine reward. Subsequent biochemical analyses of nucleus accumbens were used to examine the relationship between nicotine, ethanol, and levels of dopamine, L-dihydroxyphenylalanine (l-DOPA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in biochemical reward pathways.

In Experiment 2, subjects received nicotine and/or ethanol and pre-pulse inhibition (PPI) of the acoustic startle response was measured as a behavioral index of sensory gating. Because the acoustic startle response (ASR) and PPI have been interpreted as indices of attention (Acri, Grunberg, & Morse, 1991; Acri, Morse, Popke, & Grunberg, 1994), results indicating that ethanol can modify effects of nicotine on ASR and PPI may provide evidence that ethanol can modify nicotine's enhancing effects on attention. Similarly, results indicating that nicotine can modify ethanol's effects on ASR and PPI may provide evidence that nicotine can offset ethanol's impairing effects on attention. These results would be viewed as consistent with the suggestion that cognitive and behavioral effects of nicotine are important for the reinforcing effects of nicotine (Grunberg, Morse, & Barrett, 1983; Shiffman, Fischer, Paty, Gnys, Hickcox, et al., 1994). Subsequent biochemical analyses of striatum were performed to assess the separate and combined effects of nicotine and ethanol on biologic systems that may underlie the behavioral measures used presently. Striatum was chosen for analysis based on its established role as a mediator of PPI (Swerdlow, Caine, Braff, & Geyer, 1992).

The first section of this dissertation provides background information relevant to the hypotheses of the present experiments. First, basic pharmacologic principles of nicotine and ethanol are reviewed. Then, evidence is presented that a positive epidemiologic relationship exists between cigarette smoking and alcohol consumption. Evidence also is presented to suggest that alcohol consumption increases cigarette smoking but that cigarette smoking does not alter alcohol consumption. Next, pharmacologic factors associated with drug reward are reviewed and evidence that ethanol's effects on nicotine reward mechanisms may underlie the positive association between cigarette and alcohol consumption are presented. Finally, evidence is presented to suggest that nicotine and ethanol interact in ways that can meaningfully influence biology and behavior. Each of these findings is discussed as they relate to the present experiments and to the tendency of smokers to smoke more when they drink.

Nicotine

Nicotine (Figure 1) is a naturally-occurring alkaloid found in tobacco. The rate of nicotine absorbed from tobacco smoke varies as a function of pH. Nicotine is a weak base with a pKa of 8.0 in aqueous solution. Therefore, at pH 8.0, 50% of inhaled nicotine is ionized and 50% is non-ionized. In its non-ionized state (more alkaline conditions), nicotine will readily cross biological membranes.

Approximately 85% of inhaled nicotine is metabolized in the liver by cytochrome P-450 enzymes before being excreted through the kidneys (Julien,

1988). The primary metabolic products derived from nicotine are cotinine and nicotine-N'-oxide. Only about 17% of cotinine derived from nicotine is excreted unchanged in urine (Benowitz, Kuyt, Jacob, Jones, & Osman, 1983). The remainder is metabolized further by the liver and is excreted as secondary metabolites such as trans-3'hydroxycotinine (McKennis, Turnbull, Bowman, & Tamaki, 1962), 5'-hydroxycotinine (Bowman & McKennis, 1962), cotinine-N'-oxide (Shulgin, Jacob, Benowitz, & Lau, 1987), and cotinine methonium ion (McKennis, Turnbull, & Bowman, 1963). Nicotine-N'-oxide is excreted unchanged following intravenous administration but is reduced to nicotine and its metabolites in the gastrointestinal tract following oral administration. This reduction of nicotine-N'-oxide to nicotine within the human gastrointestinal tract is believed to result from bacterial action (Becker, Gorrod, & Jenner, 1970).

Nicotine exerts its effects in the body by binding to nicotinic acetylcholinergic receptors. Stimulation of these receptors causes central nervous system stimulation, increases in blood pressure, increases in heart rate, release of epinephrine from the adrenal medulla, and increases in the tone and activity of the gastrointestinal tract (Julien, 1988). Central nervous system stimulation by nicotine includes heightened cortical evoked potentials (Knott, 1986) and stimulation of hormone release from the hypothalamic-pituitary-stress axis (Seyler, Fertig, Pomerleau, Hunt, & Parker, 1984; Seyler, Pomerleau, Fertig, Hunt, & Parker, 1986). Nicotine also stimulates dopamine release from striatum (Blaha & Winn 1993), nucleus accumbens (Yoshida, Yokoo, Tanaka, Mizoguchi, Ishii, et al., 1993; Nisell, Nomikos, & Svensson, 1994), and the

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ventral tegmental area (VTA) (Yoshida, et al., 1993; Nisell, et al., 1994). The release of dopamine by nicotine may underlie nicotine reward and is discussed in detail in subsequent sections (see the section entitled: "Mechanisms of Nicotine Reinforcement"). Chronic administration of nicotine can result in an increase in the number of central nicotinic-cholinergic receptors (Schwartz & Kellar, 1985). This "up-regulation" of nicotinic receptors is thought to underlie nicotine tolerance (Rosecrans, Stimler, Hendry, & Meltzer, 1989; Ochoa, 1994), as well as the characteristic "withdrawal syndrome" experienced by long-time smokers who abstain from cigarettes (Rosecrans, Stimler, Hendry, & Meltzer, 1989). Although tolerance and withdrawal avoidance may motivate some individuals to continue smoking, there is no evidence suggesting that the mechanisms that underlie tolerance and withdrawal are affected by ethanol. Therefore, the present experiments focus on nicotine reward exclusively in an attempt to explain the positive epidemiologic relationship between the use of cigarettes and the use of alcohol.

The role of nicotine to maintain smoking behavior is well documented (Fant, Everson, Dayton, Pickworth, & Henningfield, 1996; USDHHS, 1988). Following the initiation of smoking, individuals regulate cigarette intake to maintain a constant level of nicotine in their body over time (USDHHS, 1987a). Over the course of each cigarette, the duration of each puff tends to decrease and/or the time between puffs tends to increase (Graham, Crouch, Levin, & Bock, 1963; Griffiths and Henningfield, 1982; Woodman, Newman, Pavia, & Clarke, 1986). This pattern suggests that smokers may smoke more

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enthusiastically at the beginning of the cigarette, when blood nicotine levels are lowest, and less enthusiastically at the end of the cigarette, at which time nicotine levels are higher. If true, then this pattern provides evidence that smokers smoke to regulate nicotine in the body.

Studies designed to examine the nicotine-regulation hypothesis have yielded results that are consistent with the notion that smokers smoke to regulate levels of nicotine. Schachter and colleagues (1977) reported a series of experiments in which nicotine availability was manipulated using behavioral and pharmacologic strategies to alter urinary pH. As noted above, acidic conditions increase ionization of nicotine thereby reducing nicotine absorption across biological membranes. This effect, in turn, slows the distribution of nicotine, decreases its reabsorption at the level of the kidney, and inhibits its effects in the body. Under acidic conditions, subjects tended to smoke more than under less acidic conditions suggesting an effort by smokers to regulate levels of bioavailable nicotine (Schachter, Kozlowski, & Silverstien, 1977; Grunberg & Kozlowski, 1986).

Additional evidence for internal nicotine regulation by smokers comes from studies using pharmacologic antagonists to inhibit effects of self-administered nicotine. Reports indicate that pretreatment with the centrally-active nicotinic receptor antagonist, mecamylamine, reduces self-reports of smoking satisfaction (Rose, Behm, Westman, & Levin, 1994) and reduces self-reported desire to smoke (Rose, Sampson, Levin, & Henningfield, 1989). These findings further support the conclusion that nicotine may be an important factor

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to motivate smoking behavior and that smokers may smoke as a means of regulating nicotine.

Ethanol

Ethanol (Figure 2) is classified pharmacologically as a general central nervous system depressant, capable of producing non-selective depression of central nervous system function. When the stomach is empty, approximately 20% of orally ingested ethanol is absorbed through the stomach with the remaining 80% absorbed through the upper intestine (Julien, 1988). If the stomach is full, then absorption is delayed. Concentration of the ethanol solution also affects absorption with more concentrated ethanol solutions being more quickly absorbed than less concentrated ethanol solutions.

The primary step in ethanol metabolism involves the oxidation of ethanol to acetaldehyde by the liver enzyme, alcohol dehydrogenase (Gilman, Rall, Nies, & Taylor, 8th ed., 1991). Acetaldehyde is then converted to acetyl coenzyme A which is oxidized through the citric acid cycle or is used in the synthesis of cholesterol, fatty acids, or other bodily constituents. Approximately 95% of ingested ethanol is metabolized in this way with the remaining 5% excreted unchanged in urine or exhaled through the lungs (Julien, 1988).

The primary effect of ethanol in the central nervous system is the graded depression of synaptic transmission. These effects may produce the characteristic "drunken" behavior of individuals who have ingested ethanol as higher cortical centers are released from the inhibitory influence of the brainstem (Julien, 1988). Peripheral effects of ethanol can include respiratory

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suppression, vasodilation, increased gastric secretion, and reduced motor coordination (Gilman, et al., 1991).

The primary mode of ethanol self-administration is oral consumption in alcoholic beverages. Eighty seven percent of all Americans over the age of 18 report having consumed alcoholic beverages at some time during their lives. Seventy three percent of those individuals report consuming alcohol during the previous twelve months and 59% report consuming alcohol during the past month (National Clearinghouse of Alcohol and Drug Information, 1995).

The Relationship Between Cigarette

and Alcohol Consumption

The positive relationship between cigarette and alcohol consumption can be examined at several levels. The "person relationship" refers to the fact that cigarette smoking and alcohol consumption tend to co-exist within individuals-- smokers tend also to be drinkers and drinkers tend also to be smokers. The "situation relationship," on the other hand, refers to the fact that individuals who are both smokers and drinkers tend to smoke more when they drink. This person-situation typology was adopted from material presented by Shiffman and Balabanis (1985). Evidence to support the existence of each type of relationship is presented below.

The "Person" Relationship Between the use of Cigarettes and Alcohol

Numerous reports suggest that a positive association exists between cigarette smoking and alcohol consumption at the person level. Friedman, Sieglaub, and Seitzer (1974) reported that 25% of normal adult smokers

sampled were heavy consumers of alcohol (three or more drinks per day), whereas only 9% of non-smokers were heavy consumers of alcohol. Reynolds, Hamas, Gallager, & Bryden (1976) reported similar results in that 45% of men who drank more than three drinks per day also were current smokers. Only 31% of those who drank fewer than 3 drinks per day also were current smokers. Among women, the smoking rates for drinkers and non-drinkers were 54% and 30%, respectively. Recently, Sobell and Sobell (1996) reported that 90 to 95% of all alcohol abusers smoke cigarettes. Together, these findings suggest that a positive correlation exists between cigarette smoking and alcohol consumption at the person level.

The "Situation" Relationship Between the use of Cigarettes and Alcohol

Just as a positive relationship between cigarette and alcohol consumption exists at the "person level," a similar relationship also is known to exist at the "situation level." Griffiths, Bigelow, and Leibson (1976) conducted a laboratory experiment to examine the causal relationship between alcohol consumption and the use of cigarettes. In a mixed sequence of laboratory sessions, subjects received either vehicle (orange juice) or vehicle with ethanol. During the ethanol test sessions, the rate of cigarette smoking increased from 26% to 117% of vehicle. Mintz, Boyd, Rose, Charuvastra, & Jarvik, (1985) conducted a similar experiment in a population of male smokers participating in a VA-sponsored methadone maintenance program. Seventy-one percent of subjects smoked more following alcohol consumption than during a comparable

test session conducted without alcohol. Together these results suggest that alcohol may act as a causal factor to increase cigarette smoking.

Just as alcohol consumption may increase cigarette consumption by people who currently smoke, alcohol consumption also poses a significant risk for relapse by people who are trying to quit smoking (Gmür & Tschopp, 1987). Borland (1990) questioned 536 smokers to determine the situations during which lapses from smoking cessation were most likely to occur. Alcohol consumption was the most common activity associated with relapse, accounting for 41% of all cessation lapses. Zimmerman, Warheit, Ulbrich, and Auth (1990) reported that drinkers not only were less likely to succeed in their attempts to quit smoking, but that drinkers also were less likely to try quitting in the first place. Together these findings suggest that alcohol consumption can increase smoking and may place individuals at risk for relapse during attempts at smoking cessation.

Directionality in the Relationship Between Cigarette Smoking and Alcohol Consumption

Results described in the previous section suggest that alcohol consumption increases cigarette smoking and that alcohol consumption may place individuals at risk for relapse during attempts at smoking cessation. It is important to note, however, the converse does not appear to be true. That is, cigarette smoking does not appear to increase alcohol consumption or to increase the risk of relapse from alcohol cessation (Bobo, Schilling, Gilchrist, & Schinke, 1986). Bobo and Gilchrist (1983) surveyed 311 alcoholic treatment

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specialists to examine professional opinions regarding cigarette smoking by recovering alcoholics. Forty-five percent of those specialists surveyed had never encouraged an alcoholic client to stop smoking. In fact, clinical opinion suggests that abstaining drinkers should be shielded from social and medical pressures to stop smoking until after they can completely abstain from alcohol (Sees & Clark, 1993).

To examine the possibility that cigarette smoking may precipitate alcohol relapse, Hurt and colleagues (1994) examined effects of nicotine dependence treatment on inpatient treatment outcomes for other addictions. If smoking were playing a role in the maintenance of drinking behavior, then one would expect higher alcohol abstinence rates among patients who quit smoking relative to those who continued to smoke. Results, however, indicated no effects of nicotine abstinence on alcohol treatment outcomes (Hurt, Eberman, Croghan, Offord, Davis, et al., 1994). Nothweir, Lando, and Bobo (1995) reported similar results in that subjects who quit smoking were no more or less likely to reduce alcohol intake than were subject who continued to smoke. The results of Hurt, et al. (1994) and Nothweir, et al. (1995) contrast sharply with the reports of Borland (1990) and Zimmerman, et al. (1990) who reported effects of alcohol consumption to precipitate smoking relapse and to inhibit attempts to quit smoking, respectively.

Several explanations are available to explain the fact that people smoke more when they drink. An argument could be made that the tendency of individuals to smoke more when they drink is, in fact, a spurious correlation.

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More specifically, it could be argued that many public places that permit individuals to smoke (i.e., restaurants, taverns) also are places that serve alcohol. Therefore, the association between cigarette and alcohol consumption may result from situational convenience and not from factors specific to cigarettes or alcohol, *per se*. Although this explanation may successfully account for the "situation relationship" between cigarettes and alcohol consumption, it fails to account for the "person relationship" between cigarette and alcohol consumption. Further, this explanation cannot account for the fact that the positive relationship between the use of cigarettes and alcohol has been recognized for generations, whereas restrictions on smoking are recent.

An alternative explanation for the positive relationship between the use of cigarettes and alcohol centers around alcohol's effects as a "social disinhibitor." The use of alcohol has been linked to the disinhibition of several types of behavior including aggression (Seto & Barbaree, 1995), sexual behavior (Crowe & William, 1989; William & Norris, 1991), and racial prejudice (Reeves & Nagoshi, 1993). Therefore, alcohol use also might disinhibit restrained cigarette smoking. However, this explanation would not account for the positive epidemiologic relationship between the use of cigarettes and alcohol among non-restraining smokers for whom smoking is not inhibited. Further, evidence indicates that the onset of the smoking habit (a time when inhibition would be expected to be at its peak) is not particularly likely to be accompanied by the use of alcohol (Friedman, Lichtenstein, & Biglan, 1985).

Because neither the spurious correlation nor the disinhibition hypothesis can successfully account for the historical and epidemiologic relationships between the use of cigarettes and alcohol, a third hypothesis is examined presently. This "complementary effects" hypothesis posits that cigarettes and alcohol have complementary effects on the body and that these complementary effects promote the co-abuse of these drugs. This is a psychobiologically-based hypothesis that focuses on psychopharmacologic effects of nicotine and ethanol to explain the common association between the use of cigarettes and alcohol. This approach minimizes the need to consider social context when considering the relationship between cigarette and alcohol consumption, thereby accounting for the historical relationship between these two drugs. In addition, the complementary effects hypothesis can account for "person," as well as "situation," relationships between the use of cigarettes and alcohol. It is important to note that the complementary effects hypothesis is intended to explain only the psychopharmacologic factors that contribute to the common association between the use of cigarettes and alcohol and is not meant to exclude social factors. Future experiment are required to account for the social factors that undoubtedly also contribute to the co-abuse of cigarettes and alcohol.

In summary, the studies reviewed in this section suggest that a positive epidemiologic relationship exists between the use of cigarettes and alcohol. Further, it appears that this relationship between alcohol consumption and cigarette smoking is unidirectional--alcohol consumption can increase cigarette

smoking but cigarette smoking does not necessarily increase alcohol consumption. In addition, alcohol consumption is a potent risk factor associated with lapses in smoking cessation. The opposite, however, does not appear to be true. Cigarette smoking is not a risk factor for relapse from alcohol cessation.

The remainder of this introduction focuses on two areas of ethanol-nicotine interactions that may be relevant to the "complementary effects hypothesis" for why people smoke more when they drink: (1) interactive effects of nicotine and ethanol on behavioral and biological indices of drug reward, and (2) interactive effects of nicotine and ethanol on behavioral indices of attention and sensory gating. Each of these interactive effects of nicotine and ethanol are discussed as they relate to the present experiments and to the tendency of smokers to smoke more when they drink.

Mechanisms of Drug Reinforcement

The search for the neural mechanisms of reward was highlighted in 1954 when Olds and Milner published the first experimental report of behavior maintained by electrical brain stimulation (Olds & Milner, 1954). Since that time, many studies have attempted to identify the pharmacologic and neurochemical substrates of brain-stimulation reward (Rolls, Rolls, Kelly, Shaw, Wood, et al., 1974; Routtenberg, 1978; see Olds & Fobes, 1981 for review), whereas others have used this information to address mechanisms that underlie drug abuse. Recently, much of the research examining mechanisms of reward has focused on central dopaminergic systems, believed to underlie brain-stimulation reward,

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to explain the reinforcing properties of drugs such as amphetamine and cocaine (Woolverton, 1986; Woolverton, & Virus, 1989; Amit & Smith, 1991; Maldonado, Robledo, Chover, Caine, & Koob, 1993). Later, these findings were used to guide investigations of the mechanisms that underlie nicotine abuse (Corrigall, 1991; Corrigall & Coen, 1991; Izenwasser, Jacocks, Rosenberger, & Cox, 1991; Corrigall, Franklin, Coen, & Clarke, 1992; Balfour & Benwell, 1993). The following section briefly reviews these reports that implicate dopaminergic systems in cocaine and amphetamine reinforcement. Then, evidence is reviewed to suggest that similar mechanisms may underlie reinforcement from nicotine. Finally, pharmacologic effects of ethanol are discussed as they pertain to the rewarding effects of nicotine.

The Role of Dopamine in Cocaine and Amphetamine Reinforcement

Treatments that are known to inhibit the activity of central dopaminergic receptors also are known to inhibit cocaine and amphetamine self-administration. Woolverton and colleagues (1986, 1989) reported that systemic administration of the D_1 antagonist, SCH 23390, inhibits operant responding for cocaine in rhesus monkeys but that systemic administration of the D_2 antagonist, pimozide, has inconsistent effects (Woolverton, 1986; Woolverton & Virus, 1989). Other experiments have found that systemic administration of the D_2 antagonist, remoxipride, can inhibit operant responding for d-amphetamine (Amit & Smith, 1991), and that central infusion of the non-specific dopamine antagonist, haloperidol, can inhibit the potentiated conditioned-reinforcement responding that is induced by pipradrol (Chu & Kelley, 1992). Because

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treatments that are known to inhibit the activity of central dopaminergic receptors also appear to inhibit cocaine and amphetamine self-administration, it seems likely that dopaminergic systems play a role to mediate rewarding effects of these psychostimulant drugs.

Subsequent research has supported this interpretation and has helped to delineate the anatomical substrates that underlie these effects. Maldonado, Robledo, Chover, Caine, & Koob (1993) injected SCH 23390 directly into nucleus accumbens (believed to be an important region for reward [Nestler, 1993]) or directly into posterior caudate nucleus of rats prior to cocaine self-administration to investigate the role of these structures in drug reward. Infusions of SCH 23390 into nucleus accumbens increased operant responding for cocaine measured 20 minutes after treatment, whereas infusions of SCH 23390 into the posterior caudate nucleus did not. Operant responding for cocaine measured three hours after treatment was unaltered by SCH 23390. The initial increases in responding measured 20 minutes after treatment with SCH 23390 may reflect a decrease in the reward value of injected cocaine. This interpretation is consistent with the reports of Woolverton et al. (1986, 1989) who reported decreases in responding for cocaine after treatment with SCH 23390. Additionally, these results suggest that dopamine activity, specifically associated with D_1 receptors in the nucleus accumbens, plays an important role to mediate reinforcing effects of cocaine (Chu & Kelley, 1992; Maldonado et al., 1993).

Mechanisms of Nicotine Reinforcement

Reports that dopaminergic systems may underlie cocaine and amphetamine reinforcement has led researchers to investigate whether similar mechanisms may underlie nicotine reinforcement. El-Bizri and Clarke (1994) reported that nicotine increases [^{3}H] dopamine release from rat striatal synaptosomes *in vitro*. This result indicates that nicotine can enhance dopaminergic activity and is consistent with the suggestion that dopamine is involved in nicotine reward. *In vivo*, nicotine enhances dopamine release from striatum (Blaha & Winn 1993), nucleus accumbens (Yoshida, Yokoo, Tanaka, Mizoguchi, Ishii, et al., 1993; Nisell, Nomikos, & Svensson, 1994), and the ventral tegmental area (VTA) (Yoshida, et al., 1993; Nisell, et al., 1994). Because dopamine receptors have been implicated as mediators of drug reward, these findings support the suggestion that dopamine is involved in reinforcing effects of nicotine. Subsequent reports indicate that effects of nicotine to elicit dopamine release from nucleus accumbens are blocked by microinjections of mecamylamine into the VTA but not by microinjections of mecamylamine into the nucleus accumbens (Nisell, et al. 1994). This finding suggests that nicotine's effects on central dopamine release are mediated by nicotinic-cholinergic receptors in the VTA but not by nicotinic-cholinergic receptors in the nucleus accumbens.

In addition to nicotine's effects to elicit dopamine release, nicotine also may inhibit dopamine uptake (Izenwasser, Jacocks, Rosenberger, & Cox, 1991; Izenwasser and Cox, 1992). This effect of nicotine to inhibit dopamine reuptake is similar to that of cocaine. Unlike cocaine, however, nicotine does not inhibit

the binding of the selective dopamine uptake inhibitor, [^{3}H]GBR 12935. This result suggests that nicotine inhibits dopamine uptake through a mechanism that does not involve the classical dopamine uptake site. Further, this result suggests a second way in which nicotine can affect central dopaminergic systems and supports the suggestion that central dopaminergic systems may mediate nicotine reward. It is important to note that a recent report by Hart and Ksir (1996), indicating an effect of nicotine to *facilitate* dopamine transporter systems, contradicts the findings of Izenwasser and colleagues (1991, 1992) or suggests that these effects may not be important *in vivo*. Future experiments, assessing effects of nicotine on dopamine uptake should help to clarify this issue.

Studies of Nicotine Self-Administration

Perhaps the most compelling evidence that dopaminergic systems are involved in nicotine reinforcement comes from studies of nicotine self-administration. Corrigall and Coen (1991) reported that subcutaneous administration of the D_1 antagonist, SCH 23390, and also of the D_2 antagonist, spiperone, inhibited nicotine self-administration by rats. Subcutaneous administration of haloperidol, a mixed D_1/D_2 receptor antagonist, had effects to reduce nicotine self-administration that approached statistical significance (p=.11). These results are similar to those of Woolverton, et al. (1986, 1989) who reported effects of SCH 23390, but not of the D_2 antagonist pimozide, to inhibit operant responding for cocaine. These results also are consistent with the decreases in smoking satisfaction reported by smokers who have been

pretreated with the nicotinic antagonist, mecamylamine (Rose, et al., 1989, 1994). Finally, these results support the suggestion that dopaminergic receptor activity is a necessary component of nicotine reward.

To investigate the anatomical substrates that underlie nicotine self-administration, Corrigall, et al. (1992) used the dopamine-specific neurotoxin, 6-hydroxydopamine, to chemically lesion dopaminergic neurons in the nucleus accumbens of rats that had been previously trained to self-administer nicotine (Corrigall, et al., 1992). As indicated in the preceding section on mechanisms of drug reward, nucleus accumbens has been implicated as an important structure mediating reward and has been implicated in the reinforcing effects of abused drugs (Nestler, 1993). As predicted, chemical lesions of nucleus accumbens significantly reduced nicotine self-administration relative to pre-lesion levels. This result is consistent with previous reports that implicate nucleus accumbens in drug reward and with the suggestion that dopaminergic activity may underlie nicotine self-administration.

As noted in the preceding section, nicotinic receptor activity, particularly in the VTA, is necessary for nicotine-induced dopamine release from nucleus accumbens. To investigate the role of nicotinic receptor activity in nicotine self-administration, Corrigall, Coen, and Adamson (1994) infused the nicotinic antagonist, dihydro-β-erythrodine (DHβE) directly into nucleus accumbens or directly into the VTA. Microinfusions of DHβE into VTA reduced operant responding for nicotine, whereas similar infusions into nucleus accumbens were without effect. This result is consistent with reports that nicotine's effect to

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increase dopamine release from nucleus accumbens is mediated by nicotinic

receptors in the VTA. Additionally, these results suggest that nicotine-induced

dopamine release may mediate nicotine self-administration. If this action is

altered by ethanol, then it may help to explain the particularly rewarding effects

of smoking cigarettes after consuming alcohol.

The Role of Serotonin in Nicotine Reward

Although available evidence suggests that central dopaminergic systems

play the most important role to mediate nicotine reinforcement, it is unlikely that

nicotine reinforcement is mediated by nicotinic cholinergic and dopaminergic

activity exclusively. Substantial evidence suggests that other transmitter

systems, most notably serotonergic, are sensitive to effects of nicotine

(USDHHS, 1988) and can mediate nicotine's effects on dopamine.

Serotonergic receptors (i.e., 5-HT$_3$) are highly concentrated in the nucleus

accumbens (Kilpatrick, Jones, & Tyers, 1987) and activation of 5-HT$_3$ receptors

by serotonergic agonists such as 2-methylserotonin (Jiang, Ashby, Kasser, &

Wang, 1990) and 1-phenylbiguanide (Chen, van Praag, & Gardner, 1991) dose-

dependently enhance dopamine release in this area. Because dopaminergic

activity in nucleus accumbens is important for nicotine reward, findings that

serotonergic agonists increase dopamine release in this area suggest a

mechanism by which serotonergic systems may influence nicotine reward.

Several experiments have attempted to equate serotonergically-mediated

dopamine release with serotonergically-mediated nicotine self-administration,

with decidedly mixed results. Corrigall and Coen (1994) reported that the 5-HT$_3$

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antagonist, ICS 205-930, weakly inhibits nicotine self-administration in rats, but that the 5-HT$_3$ antagonist MDL 72222, has no effect. In humans, the 5-HT$_3$ antagonist, ondansetron, has no effect on cigarette consumption (Zancy, Appelbaum, Lichtor, & Zaragoza, 1993), whereas several 5-HT agonists (5-hydroxytryptamine, quipazine, and ipsapirone) have been shown to attenuate oral nicotine self-administration by non-human primates (Opitz & Weichler, 1988). Additional research is necessary to fully understand the role of serotonin to mediate nicotine's effects on dopamine and to fully understand the role of serotonin in nicotine reinforcement.

To summarize, it appears that central dopaminergic activity, particularly in the nucleus accumbens and ventral tegmental area, plays an important role in nicotine reinforcement. Treatments that inhibit biologic effects of nicotine (e.g., mecamylamine, SCH 23390) also inhibit nicotine self-administration. It is known that nicotine self-administration and nicotine-induced dopamine release are antagonized by administration of mecamylamine into the VTA but not by administration of mecamylamine into the nucleus accumbens. These findings suggest that nicotine reward may be mediated by nicotinic receptor activity in the VTA (which causes an efflux of dopamine from nucleus accumbens), but not by nicotinic receptor activity in nucleus accumbens *per se*. Serotonergic systems, which are activated by nicotine (Robiro, Bettiker, Bogdannov, & Wurtman, 1993; Westfall, Grant, & Perry, 1983; Yu & Wecker, 1994), may play a role to modulate nicotine-induced dopamine release in the nucleus

accumbens and the VTA, thereby modulating nicotine reward. The role of serotonin in nicotine self-administration remains unclear.

Effects of Ethanol on Mechanisms of Nicotine Reward

In the preceding section, it was suggested that pharmacologically-induced decreases in nicotine's biological effects are associated with decreases in nicotine self-administration. Because decreases in nicotine's biological effects are associated with decreases in nicotine self-administration, it is reasonable to postulate that increases in nicotine's biological effects would increase nicotine self-administration. In the next section, evidence is presented that ethanol can potentiate nicotine's biological effects, perhaps resulting in increases in nicotine self-administration. Particular attention is paid to dopaminergic and serotonergic systems because these are most likely to affect nicotine reinforcement and, therefore, have the greatest relevance to the question of why people smoke and drink concurrently. The available research indicates that ethanol: (1) slows the conversion of nicotine to its primary metabolite, cotinine; (2) increases the rate of dopamine and serotonin synthesis; and (3) slows the rate of dopamine and serotonin metabolism. Each of these effects of ethanol is discussed in detail below.

Ethanol Slows the Conversion of Nicotine to Cotinine

Several lines of evidence suggest that ethanol's pharmacologic effects may enhance the reward from subsequently administered nicotine. It is known, for example, that ethanol slows the conversion of nicotine to its primary

metabolite, cotinine. Domdey-Bette and Shüppel (1988) reported significantly slower conversion of nicotine to cotinine in livers treated with 22-40 mM ethanol compared to livers that had not been treated with ethanol. Although the mechanism responsible for this effect is unknown, it is likely that ethanol inhibits nicotine metabolism by competing for hepatic cytochrome P-450 enzymes. These enzymes comprise the primary metabolic pathway for nicotine (Peterson, Trevor, & Castagnoli, 1987) and are known to be responsive to ethanol (Warner, Strömstedt, Wyss, & Gustafsson, 1993; Nanji, Zhao, Lamb, Dannenberg, Sadrzadeh, et al., 1994; Warner & Gustafsson, 1994). Because cotinine is largely inactive relative to nicotine (Dar, Li, & Bowman, 1993), ethanol's effect to inhibit the conversion of nicotine to cotinine may prolong or intensify rewarding effects of self-administered nicotine. Because decreases in effects of nicotine are associated with decreases in nicotine self-administration (Corrigall, et al., 1994), this potential effect of ethanol to *increase* effects of nicotine may be useful to explain increased nicotine self-administration by smokers who drink alcohol.

Ethanol Increases the Rate of Dopamine and Serotonin Synthesis

A second means by which ethanol may enhance nicotine reward is through its effects to enhance the synthesis rate of dopamine and serotonin. As noted in the preceding section on nicotine self-administration, increases in the activity of dopaminergic and serotonergic systems are associated with increases in nicotine self-administration and reward. Treatments that increase the bioavailability of these transmitters, by increasing the rate of their biosynthesis,

may also increase the reinforcing effect of subsequently administered nicotine. Waldeck (1974) examined effects of ethanol on dopamine synthesis by measuring [^{3}H] dopamine overflow following injection of ethanol and [^{3}H] tyrosine. Tyrosine is the initial substrate for all catecholamine synthesis and, therefore, provides a marker for the measurement of synthesized dopamine. There is a significant increase in [^{3}H] dopamine (presumably synthesized from the previously-administered [^{3}H] tyrosine) in subjects treated with 4.0 g/kg ethanol (IP) relative to subjects not treated with ethanol. This finding suggests that ethanol can increase the rate of dopamine synthesis and suggests a mechanism by which ethanol may increase rewarding effects of subsequently-administered nicotine. Specifically, ethanol-induced increases in the rate of dopamine synthesis may amplify nicotine's effects by potentiating nicotine-induced dopamine release. Baizer, Masserano, and Weiner (1981) further examined effects of ethanol on dopamine synthesis, and reported increases in tyrosine hydroxylase in striatum, locus coeruleus, and frontal cortex of rats following an acute injection of ethanol. Tyrosine hydroxylase is the rate-limiting enzymatic step in the synthesis of dopamine. Therefore, increases in tyrosine hydroxylase activity suggest increases in the rate of dopamine synthesis. Sze (1977) reported similar effects of dietary ethanol to increase tryptophan hydroxylase activity in whole brain homogenate. Because tryptophan hydroxylase is the rate-limiting enzymatic step in the synthesis of serotonin, increases in tryptophan hydroxylase activity may suggest increases in the rate

of serotonin synthesis. Effects of ethanol to increase serotonin synthesis may, in turn, play a role to increase the reinforcing effects of nicotine.

A third means of examining ethanol's effects on dopamine synthesis is to examine levels of the dopamine precursor, dihydroxyphenylalanine (l-DOPA), following treatment with ethanol and with the l-aromatic amino acid decarboxylase inhibitor, NSD 1015. Treatment with NSD 1015 blocks the conversion of l-DOPA to dopamine by inhibiting the catalytic enzyme, DOPA decarboxylase. Accumulation of l-DOPA following treatment with NSD 1015 provides an index of activity in the dopamine synthesis pathway. Blomqvist, Engel, Nissbrandt, and Söderpalm (1993) reported that rats treated with dietary ethanol (following treatment with NSD 1015) had higher l-DOPA concentrations in the limbic areas including nucleus accumbens, olfactory tubercles, amygdala, and septum, than did subjects that had not been treated with ethanol. This result is consistent with the suggestion that ethanol increases the rate of dopamine synthesis and also with the suggestion that ethanol may sensitize mechanisms of nicotine reward.

<div align="center">Ethanol Inhibits Dopamine and Serotonin Metabolism</div>

An additional way in which ethanol may potentiate nicotine reward is to inhibit dopamine and serotonin metabolism. This effect may increase the length of time that serotonin and dopamine are available to interact with receptors, thereby prolonging the rewarding effects mediated by these neurotransmitters. Blanchard, Steindorf, Wang, and Glick (1993) reported effects of ethanol to increase concentrations of dopamine in dialysate from nucleus accumbens

without altering concentrations of the dopamine metabolites,

dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) . Reports that

extracellular concentrations of these metabolites are unchanged, despite

increases in extracellular concentrations of dopamine, suggest that ethanol may

inhibit the rate of dopamine metabolism. Yoshimoto, McBride, Lumeng, and Li

(1992) reported similar effects of ethanol to increase extracellular dopamine

with a concomitant decrease in extracellular DOPAC. This finding further

supports the suggestion that ethanol can slow the metabolism of dopamine.

Ethanol's effects on serotonin metabolism appear to be similar to its

effects on dopamine metabolism. Specifically, ethanol increases extracellular

concentrations of serotonin without altering extracellular levels of the serotonin

metabolite, 5-hydroxyindolacetic acid (5-HIAA) (Yoshimoto, Komura, &

Kawamura, 1992). Metabolites of ethanol, such as 1-methyl-6,7-dihydroxy-

1,2,3,4,-tetrahydroisoquinoline, also increase extracellular concentrations of

serotonin while simultaneously decreasing extracellular concentrations of 5-

HIAA (Nakahara, Maruyama, Hashiguti, & Naoi, 1994). Because the increases

in extracellular serotonin that are produced by ethanol and ethanol metabolites

are not accompanied by increases in extracellular 5-HIAA, these results suggest

that ethanol-induced increases in serotonin synthesis and release may be

accompanied by a concomitant decrease in serotonin metabolism. This effect,

in turn, may increase the availability of serotonin, thereby increasing the effects

of serotonergic systems to mediate nicotine reward.

Although the mechanisms that underlie effects of ethanol on dopamine and serotonin metabolism are uncertain, available evidence suggests that ethanol may inhibit the metabolism of dopamine and serotonin by inhibiting the activity of monoamine oxidase-B (MAO-B). MAO-B is the metabolizing enzyme responsible for the conversion of dopamine to DOPAC and for the conversion of serotonin to 5-HIAA. Rommelspacher, May, Dufeu, and Schmidt (1994) reported that levels of monoamine oxidase-B are significantly reduced in the platelets of alcoholics during chronic intoxication and in normal controls during acute intoxication. May and Rommelspacher (1994) reported a similar effect of ethanol to inhibit MAO-B. These data indicate that ethanol can inhibit the activity of MAO-B, suggesting a possible mechanism by which ethanol may slow the metabolism of dopamine and serotonin.

Considering the available data, it appears that ethanol may enhance the rewarding effects of nicotine by: (1) slowing the conversion of nicotine to cotinine; (2) increasing the rate of dopamine and serotonin synthesis; and (3) inhibiting the rate of dopamine and serotonin metabolism. Because both dopamine and serotonin have been implicated in nicotine-induced reward, these effects of ethanol may have important implications for why people smoke more when they drink. Specifically, ethanol may increase the rewarding effects of nicotine by slowing its conversion to the relatively inactive nicotine metabolite, cotinine. Nicotine-induced dopamine and serotonin release, known to be important mediators of nicotine reward, may then be potentiated by ethanol-induced increases in dopamine and serotonin synthesis. Additionally, these

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released neurotransmitters may exert more-powerful, longer-lasting effects as a result of ethanol-induced suppression of dopamine and serotonin metabolism. The net result, therefore, may be an effect of ethanol to increase the rewarding effects of subsequently-administered nicotine. Previous studies have reported that it is possible to reduce nicotine self-administration by reducing the activity of nicotinic, dopaminergic, and serotonergic receptors (Corrigall & Coen, 1991; Corrigall, et al., 1992 Corrigall & Coen, 1994; Corrigall, et al., 1994). It is reasonable to postulate, therefore, that factors that have opposite effects (i.e., factors that *increase* the activity of nicotinic, dopaminergic, and serotonergic receptors) may *increase* nicotine self-administration. Specifically, it is possible that effects of ethanol to: (1) slow the conversion of nicotine to cotinine; (2) increase the rate of dopamine and serotonin synthesis; and (3) inhibit the rate of dopamine and serotonin metabolism, may effectively agonize nicotinic reward systems, thereby increasing nicotine self-administration by smokers.

Examining Ethanol's Effects on Nicotine Reward

in Experiment 1

Experiment 1 examined effects of ethanol that may potentiate nicotine reward using the conditioned place preference paradigm. In the conditioned place preference paradigm, a drug is repeatedly paired with a distinct set of environmental cues. Over the course of several drug-environment pairings, an association is formed between the effects of the drug and the contextual cues associated with the conditioning environment. Drugs that are known to have reinforcing properties, such as cocaine and amphetamine, will condition a preference for the environments with which they are paired (Reicher & Holman, 1977; Mucha, Van Der Kooy, O'Shaughnessy, & Bucenieks, 1982; Carr & White, 1983). Drugs that are known to have aversive effects, on the other hand, such as lithium chloride, will condition an aversion to the environment with which they are paired (Mucha, et al., 1982)[1]. Studies of nicotine-induced place preference indicate that repeated exposure to nicotine will condition preference for the nicotine-paired environment (Fudala, Teoh, & Iwamoto, 1985; Carboni, Acquas, Leone, & Di Chiara, 1989; Acquas, Carboni, Leone, & Di Chiara, 1989).[2] Therefore, the conditioned place preference paradigm may be useful to

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[1]The terms "preference" and "aversion" are used to describe the proportion of time that an animal spends in a drug-paired vs. a non-drug-paired environment. They should be taken as *indices* of motivation and not as a direct *measure* of motivation in these non-human subjects.

[2]Pilot data, collected using the procedures outlined for Experiment 1, have verified effects of nicotine to condition place preference in Sprague-Dawley rats.

study effects of ethanol on nicotine reward. To examine biological correlates associated with nicotine and alcohol administration, levels of dopamine, the dopamine precursor, l-DOPA, the intracellular dopamine metabolite, DOPAC, and the extracellular dopamine metabolite, HVA were measured in the nucleus accumbens immediately following administration of nicotine and/or ethanol.

Behavioral and Physiologic Interactions

of Nicotine and Ethanol

An additional area of nicotine/ethanol interaction that may be relevant to the question of why smokers smoke more when they drink, involves effects of nicotine and ethanol on cognitive-motor performance, psychomotor stimulation, and general physiologic arousal. It has been suggested that nicotine may offset some of the negative effects of ethanol and that these effects of nicotine may be responsible for the positive association between cigarette smoking and alcohol consumption (Shiffman, Fischer, Paty, Gnys, Hickcox, et al., 1994). In mice, nicotine has been shown to attenuate ethanol-induced motor incoordination (Dar, Li, & Bowman, 1993; Dar, Bowman, & Li, 1994) and to attenuate ethanol's effects on respiration (Burch, De Fiebre, Marks, & Collins 1988) and body temperature (Burch, et al. 1988; Collins, Burch, De Fiebre, & Marks, 1988). In humans, cigarette smoking can offset the impairing effects of ethanol on choice reaction time (Lyon, Tong, Leigh, & Clare, 1975), motor function (Kerr, Sherwood, & Hindmarch, 1991), and vigilance performance (Michael & Bättig, 1989). To the extent that the smoker perceives these

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impairing effects of ethanol as aversive, then (s)he may smoke more when (s)he drinks in an attempt to offset these effects.

An alternative behavioral explanation for the positive association between cigarette smoking and alcohol consumption is that ethanol may potentate effects of nicotine that the smoker finds rewarding. Schaefer and Michael (1992) reported that nicotine increased locomotor activity in mice by roughly 70% when administered alone, and by well over 100% when administered with ethanol. To the extent that smokers smoke to experience the stimulatory effects of nicotine, these data suggest a clear rationale for smoking more when they drink. Specifically, subjects may have learned that the nicotine from cigarettes will be more beneficial to them following alcohol consumption. Lyon and colleagues (1975) reported effects of ethanol on decision time and motor speed in deprived smokers, smokers who were not deprived, and nonsmokers. Smokers who were not deprived had faster motor and decision times than did either of the other two groups. Interestingly, however, the fastest motor and decision times were recorded in non-deprived smokers who had also received alcohol. These data suggest that nicotine alone has beneficial effects on performance that are potentiated by ethanol. Additionally, these data support the notion that smokers smoke more when they drink as a means of augmenting the behavioral effects of nicotine.

Though these findings suggest that physiologic and behavioral interactions may exist between nicotine and ethanol, it is important to note that these interactions may not be universal. Quantitative genetic analyses have

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revealed a major genetic component that influences smoking behavior (Heath, Madden, Slutske, & Martin, 1995) and a common genetic latent factor underlying the observed associations between the use of cigarettes and alcohol (Swan, Carmelli, & Cardon, 1996). Luo, et al. (1994) investigated the possibility that genetic characteristics may underlie interactive effects of nicotine and ethanol by examining differential effects of nicotine and ethanol in long-sleep and short-sleep strains of mice. These strains of mice have been selectively bred to be differentially sensitive to the sleep-inducing effects of ethanol. Only the long-sleep mice exhibited tolerance to ethanol's effects on open-field activity, body temperature, and sleep time. Additionally, only the long-sleep mice exhibited measurable tolerance to subsequent injections of nicotine. These results suggest that similar genetic characteristics may underlie sensitivity to nicotine and ethanol. In a similar experiment, Gordon, Meehan, and Schechter (1993b) examined the locomotor-stimulating effects of nicotine in two strains of rats selectively bred to prefer or to not prefer alcohol. Acute nicotine treatment significantly enhanced locomotor activity in the alcohol-preferring strain, but either attenuated or did not change the locomotor activity in the alcohol non-preferring strain. Preferring and non-preferring rats also differ in their sensitivity to the discriminative stimulus effects of nicotine with alcohol-preferring rats having greater sensitivity to the discriminative stimulus effects of nicotine than non-preferring rats (Gordon, Meehan, & Schechter, 1993a). Mice bred to be differentially sensitive to the locomotor-stimulating effects of ethanol, however, do not differ in their sensitivities to the locomotor-stimulating effects of

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nicotine (Phillips, Burkhart-Kasch, Gwiazdon, & Crabbe, 1992) suggesting that the genetic link between alcohol and nicotine sensitivity is not absolute.

Examining Effects of Nicotine and Ethanol on Psycho-Motor Performance in Experiment 2

In Experiment 2, effects of nicotine and ethanol on psychomotor functioning were assessed using the acoustic startle response (ASR) and pre-pulse inhibition of the acoustic startle response (PPI) as an animal model of sensory gating. The acoustic startle response (ASR) is a simple reflex behavior that occurs in response to abrupt sensory stimuli. The neural circuitry that underlies this response is thought to include the auditory nerve, the ventral cochlear nucleus, nuclei of the lateral lemniscus, nucleus reticularis pontis caudalis, spinal neuron, and lower motor neuron (Davis, Gendelman, Tischler, & Gendelman, 1982). For several reasons, this behavior provides an excellent animal model to study effects of drugs on behavior. First, the acoustic startle response can be elicited using identical stimulus parameters in humans and in animals, thereby enabling cross-species generalizations to be made (Swerdlow, Caine, Braff, & Geyer, 1992). Second, although the primary neural circuitry that underlies the acoustic startle response involves structures at, or below, the midbrain, the response exhibits several types of plasticity that are thought to involve "higher" brain structures (Swerdlow, et al., 1992). One such form of plasticity is known as pre-pulse inhibition (PPI) and will be discussed in detail on the following two pages.

Numerous studies have reported effects of drugs to alter the amplitude of

ASR. ASR is increased by dopaminergic agents such as apomorphine (Davis,

1988), d-amphetamine (Davis, Svensson, & Aghajanian, 1975; Kokkinidis &

Anisman, 1978), and cocaine (Harty & Davis, 1985), and is inhibited by ethanol

(Pohorecky, Cagan, Brick, & Jaffe, 1976). More recently, studies have

examined effects of nicotine on ASR (Acri, et al., 1991; Acri, et al., 1994; Popke,

et al., 1994). Acri, et al. (1991) reported effects of chronic nicotine (12

mg/kg/day; administered by osmotic minipump) to increase ASR amplitude

relative to saline controls. Others have reported similar effects of acute nicotine

(0.01 mg/kg nicotine; SC) to increase ASR amplitudes relative to controls (Acri,

et al., 1994; Popke, et al., 1994). These results have been interpreted as

reflecting changes in attentional processes produced by nicotine (Acri, Morse,

Popke, & Grunberg, 1994).

Pre-pulse inhibition (PPI) refers to the reduction in startle amplitude that

occurs when a startling stimulus is briefly preceded by a non-startling tone.

Because the interval between the presentation of the pre-pulse tone and the

presentation of the startle stimulus is too short to permit volitional processing,

pre-pulse inhibition is thought to reflect an innate "gating" mechanism relating to

sensory-motor function (Swerdlow, et al., 1992). This supposition has led to the

use of the pre-pulse inhibition paradigm to study attentional processes (Acri, et

al., 1994) and to model the time-dependent sensory gating deficits associated

with schizophrenia (Swerdlow, Braff, Geyer, & Koob 1986).

Studies of nicotine's effects on PPI have yielded results that are consistent with nicotine's enhancing effects on attention. Specifically, it has been reported that acute (0.01 mg/kg; SC) (Acri, et al, 1994; Popke, et al., 1994) and chronic (12 mg/kg/day; administered by osmotic minipump)(Acri, 1994) nicotine each enhance pre-pulse inhibition relative to controls. Nicotine administration increases the extent to which the startle response is reduced when the startling stimulus is preceded by a non-startling tone.

The neural circuitry that underlies PPI is thought to include hippocampal efferents to the striatum and striatal GABAergic efferents to the ventral pallidum (Swerdlow, et al., 1992). Pallidal efferents may impinge on the primary acoustic startle circuit at the level of the mesencephelon. Additional modulation of PPI occurs in the striatum and is thought to involve primarily D_2 receptors (Swerdlow, et al., 1992).

SUMMARY OF DATA BASED ON THE LITERATURE REVIEWED

A positive relationship exists between the consumption of cigarettes and the consumption of alcohol. Further, it seems clear that this relationship is unidirectional--alcohol consumption increases smoking but smoking does not necessarily increase alcohol consumption. Nicotine reward relies heavily on dopaminergic activity in the nucleus accumbens and on nicotinic activity in the ventral tegmental area (VTA). Treatments that inhibit dopaminergic activity in the nucleus accumbens, or block nicotinic activity in VTA, can inhibit nicotine-induced dopamine release and can attenuate nicotine self-administration. The role of serotonergic systems in nicotine reward is less clear but it is known that

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serotonergic agonists potentiate dopamine release in nucleus accumbens and that serotonergic antagonists can attenuate nicotine self-administration.

Because conditions that inhibit the function of nicotine reward systems (such as nicotinic, dopaminergic, or serotonergic antagonists) reduce nicotine self-administration, conditions that enhance the function of nicotine reward systems (such as ethanol) might increase nicotine self-administration. Effects of ethanol to enhance the function of these physiologic reward systems may result from its effects to: (1) slow the conversion of nicotine to the relatively inactive nicotine metabolite, cotinine; (2) increase the rate of dopamine and serotonin synthesis by increasing tyrosine and tryptophan hydroxylase activities, respectively; and (3) to slow the rate of dopamine and serotonin metabolism by inhibiting the metabolizing enzyme, monoamine oxidase-B. Because these transmitters are known to play an important role in nicotine reward, effects of ethanol to increase the efficiency of their action may make self-administered nicotine more rewarding to smokers when they drink.

Behavioral data have been reviewed to suggest that some effects of ethanol can be modified or attenuated by nicotine and that some effects of nicotine can be modified or potentiated by ethanol. Specifically, nicotine can attenuate impairing effects of ethanol on reaction time (Lyon, et al., 1975), motor performance (Kerr, et al., 1991), and vigilance performance (Michael & Bättig, 1989) in humans, and can attenuate depressing effects of ethanol on respiration (Burch, et al., 1988) and body temperature (Burch, et al., 1988; Collins, et al., 1988) in mice. Ethanol, in turn, can potentiate nicotine's

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enhancing effects on locomotor activity in mice (Schaefer & Michael, 1992) and on decision time and motor performance in humans (Lyon, et al., 1975). Together, these effects of nicotine and ethanol may combine to increase the reinforcing effect of smoking and drinking concurrently.

The present dissertation research had several purposes. The first purpose was to determine whether an acute injection of ethanol would modify behavioral indices of nicotine reward using the conditioned place preference paradigm. The second purpose was to determine whether impairing effects of acute ethanol on ASR and PPI would be offset by effects of acute nicotine or whether enhancing effects of acute nicotine on ASR and PPI would be enhanced by ethanol. The third purpose of the present dissertation research was to examine separate and combined effects of nicotine and ethanol on biochemical indices that are relevant to present behavioral measures. In Experiment 1, effects of an acute injection of nicotine and/or ethanol on levels of l-DOPA, dopamine, DOPAC, and HVA in the nucleus accumbens were measured. In Experiment 2, effects of an acute injection of nicotine and/or ethanol on levels of l-DOPA, dopamine, DOPAC, and HVA in the striatum were measured. It is important to note that Experiments 1 and 2 were designed to test separate, but related, components of the "complementary effects" hypothesis. Although these experiments were independent of each other, both have relevance to the question of why people smoke more when they drink. The purpose of designing these experiments independently (as opposed to

serially) was to ensure that the integrity of Experiment 2 was unaffected by the outcome of Experiment 1 and *vice versa*.

SPECIFIC HYPOTHESES OF THE PRESENT EXPERIMENTS

Eight major hypotheses were tested in the present experiments using male Sprague-Dawley rats. Hypotheses 1, 3, and 4 are replications of previous findings. Hypotheses 2, 5, 6, 7, and 8 are new hypotheses.

Experiment 1

Hypothesis 1. In a conditioned place preference situation, subjects will exhibit a preference for an environment that has been paired with nicotine relative to an environment that has been paired with saline.

Rationale. Previous reports and pilot data indicate effects of nicotine to condition place preference in male Sprague-Dawley rats using the nicotine dose and administration route used presently (Fudala, et al., 1985; Carboni, et al., 1989; Acquas, et al., 1989). Therefore, subjects should display preference for an environment that has been paired with nicotine relative to an environment that has been paired with saline.

Hypothesis 2. Acute administration of ethanol will potentiate conditioned place preference for nicotine.

Rationale. Because the conditioned place preference paradigm has been used to study reinforcing effects of abused drugs (Reicher & Holman, 1977; Mucha, et al., 1982; Carr & White, 1983), effects of nicotine to condition place preference in rats are viewed as evidence of nicotine reward. Evidence reviewed in the introduction of this proposal indicates that ethanol may potentiate nicotine reward. This potentiation of nicotine

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reward by ethanol should be reflected as increased place preference conditioning to nicotine.

Experiment 2

Hypothesis 3. A moderate dose of nicotine (0.01 mg/kg; SC) will increase the amplitude of the acoustic startle response (ASR) and will increase pre-pulse inhibition (PPI) relative to saline controls.

Rationale. Previous reports indicate that acute administration of 0.01 mg/kg nicotine increases ASR and PPI (Acri, et al., 1994; Popke, et al., 1994). Therefore, acute administration of 0.01 mg/kg nicotine should increase ASR and PPI relative to controls.

Hypothesis 4. A high dose of nicotine (0.5 mg/kg; SC) will not alter ASR or PPI relative to saline controls.

Rationale. Previous reports indicate that effects of 0.5 mg/kg nicotine on ASR and PPI reflect the descending limb of an inverted U-shaped dose-effect of nicotine and that these effects produce levels of ASR and PPI that are indistinguishable from controls (Acri, et al., 1994). Therefore, acute administration of 0.5 mg/kg nicotine should produce levels of ASR and PPI that are indistinguishable from controls.

Hypothesis 5. Ethanol will decrease ASR and PPI in a linear dose-response fashion.

Rationale. Previous reports indicate that acute administration of ethanol impairs performance on indices of psychomotor function (Pohorecky, et al., 1976). Because ASR and PPI have been proposed as indices of

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psychomotor function, acute administration of ethanol should reduce ASR and PPI.

Hypothesis 6.   Nicotine pre-treatment will significantly attenuate ethanol's effects to decrease ASR and PPI.

Rationale.   Previous reports indicate that nicotine pre-treatment can offset the impairing effects of ethanol on indices of psychomotor functioning (Lyon, et al., 1975; Michael & Bättig, 1989; Kerr, et al., 1991).   Because the acoustic startle paradigm has been proposed as an animal model of psychomotor function (Swerdlow, et al., 1986; Acri, et al., 1991), nicotine pretreatment should attenuate ethanol's impairing effects on ASR and PPI.

Hypothesis 7.   Ethanol pre-treatment will potentiate nicotine's effects to increase ASR and PPI.

Rationale.   Previous reports indicate that ethanol pre-treatment can potentiate the enhancing effects of nicotine on indices of psychomotor functioning (Lyon, et al., 1975; Schaefer & Michael, 1992).   Because the acoustic startle paradigm has been proposed as an animal model of psychomotor function (Swerdlow, et al., 1986; Acri, et al., 1991), nicotine pretreatment should potentiate nicotine's enhancing effects on ASR and PPI.

Hypothesis 8.   Acute administration of nicotine and ethanol together will result in dopamine levels in striatum that are greater than those observed when nicotine is administered alone.

Rationale. Evidence reviewed in the introduction indicates that ethanol can

increase the function of dopaminergic mechanisms, particularly those

associated with dopamine synthesis, release, and metabolism. This

ethanol-induced potentiation of dopaminergic mechanisms should be

reflected in additive effects of nicotine and ethanol on dopamine levels in

striatum.

EXPERIMENT 1: EFFECTS OF ACUTE ETHANOL ON NICOTINE-INDUCED CONDITIONED PLACE PREFERENCE IN MALE RATS

Overview

The purpose of this experiment was to examine ethanol's effects on nicotine-induced conditioned place preference in male rats. The conditioned place preference paradigm has been used extensively to study rewarding effects of drugs, including nicotine and ethanol in separate experiments (Fudala, Teoh, & Iwamoto, 1985; Fudala & Iwamoto, 1986; Acquas, Carboni, Leone, & Di Chiara, 1989; Carboni, Acquas, Leone, & Di Chiara, 1989). Repeated pairings of the drugs of interest with a distinct set of environmental stimuli can result in conditioned place preference (or, in some cases, aversion) to those particular stimuli. Preference for the drug-paired environment in a free-choice situation is taken as evidence that the drug of interest is reinforcing. Aversion to the drug-paired environment in a free-choice situation is taken as evidence that the drug is aversive.

In the present experiment, the rewarding effects of nicotine were assessed using the conditioned place-preference paradigm. The effects of ethanol to potentiate nicotine reward also was assessed. Nicotine-induced place preference was assessed using a two-chambered shuttle-box avoidance system (San Diego Instruments, San Diego, CA) (see Table 1 for timeline). On days 1-3 of the experiment, subjects were given simultaneous access to both chambers of the shuttle-box to determine each subjects "unbiased" place preference. On days 4-8 of the experiment, place preference for nicotine was

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established by pairing acute injections of nicotine with one of the shuttle-box

chambers and by pairing acute injections of saline with the other. Pairings were

such that half of the subjects received nicotine in their preferred chamber,

whereas the other half received nicotine in their non-preferred chamber.

Control subjects that received saline only during conditioning were paired with

subjects that received nicotine in a matched case-control design. This

procedure ensured that every nicotine-treated subject was paired with a

comparable control subject that received saline in the same chamber (i.e.,

preferred or non-preferred). Table 2 provides a description of experimental

conditions. Table 3 provides a listing of case-control pairings. On day 9,

subjects were again given access to both chambers. During this free-choice

period, the amount of time spent in the drug-paired (i.e., conditioned) chamber

relative to the amount of time spent in the saline-paired (i.e., non-conditioned)

chamber was measured to assess place-preference conditioning induced by

nicotine. This difference in time was used as a behavioral index of nicotine

reward. On day 10, subjects received an acute injection of either ethanol,

saline, or ethanol + nicotine and were given access to both shuttle-box

chambers. If ethanol increased the proportion of time spent in the nicotine-

paired chamber relative to the saline-paired chamber, then it would suggest that

nicotine reward was enhanced by ethanol. If ethanol decreased the proportion

of time spent in the nicotine-paired chamber relative to the saline-paired

chamber, then it would suggest that nicotine reward was reduced by ethanol.

Immediately after testing on day 10, subjects were sacrificed by decapitation.

Nucleus accumbens were removed from whole brain and were frozen at -80° C.

High-Performance Liquid Chromatography (HPLC) was used to determine

levels of l-DOPA, dopamine, DOPAC, and HVA in nucleus accumbens.

Methods

Subjects and Housing

Subjects were 96 male Sprague-Dawley rats (Charles River Labs,

Willmington, MA) weighing 200 g and all roughly 7 weeks old at the beginning of

the experiment. The use of non-human subjects allowed pharmacologic

manipulations and experimental procedures that were not possible in human

subjects. Sprague-Dawley rats are commonly used in the published literature.

Therefore, the use of Sprague-Dawley rats presently allowed comparisons

between the results of the present experiment and results of experiments run

previously using the same conditioned place preference paradigm (Fudala, et

al., 1985; Fudala & Iwamoto, 1986; Acquas, et al., 1989; Carboni, et al., 1989).

Age of subjects also was selected to be consistent with published reports and to

allow comparison between results of the present experiment(s) and results of

experiments run previously using the same conditioned place preference

paradigm. A sample of 96 subjects was sufficient to include 32 subjects in each

between-subjects comparison in this experiment (16 subjects in each

experimental group). This sample size affords an 80% probability of detecting

significant experimental effects given an alpha level of 0.05 and an experimental

effect size of 0.5 (Cohen & Cohen, 1988). This effect size was determined

based on published reports using similar conditioned place preference

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paradigms (Fudala, et al., 1985; Fudala & Iwamoto, 1986; Acquas, et al., 1989; Carboni, et al., 1989) .

Animals were housed individually in 35.6 cm x 15.2 cm x 20.3 cm cages with absorbent Pine-Dri, wood-chip bedding. Animals were maintained under a 12 h light/dark cycle (lights on at 0700) at approximately 23 degrees C and 50% relative humidity. Water and laboratory chow (Teclab 7000) were available continuously in the home cage. The housing conditions described above are consistent with previous reports using behavioral measures to study effects of nicotine (Acri, Morse, Popke, & Grunberg, 1994; Grunberg, Acri, & Popke, 1994; Popke & Grunberg, 1994).

Nicotine administration

During place-preference conditioning (days 4-8, inclusive), subjects received injections of either saline or 0.8 mg/kg nicotine base administered subcutaneously (SC) in volumes ranging from 0.2 to 0.4 ml. Physiological saline (0.9% NaCl) was used to prepare the nicotine solutions from nicotine dihydrochloride and was used as the control solution. This dose of nicotine and volume of injection is consistent with previous reports using SC nicotine in comparable conditioned place-preference paradigms with nicotine (Fudala, et al., 1985; Fudala & Iwamoto, 1986; Acquas, et al., 1989; Carboni, et al., 1989).

Ethanol administration

Subjects received injections of either 1.0 g/kg ethanol or saline administered intraperitoneally (IP) in volumes ranging from 1.5 to 3.0 ml. Physiological saline (0.9% NaCl) was used to prepare solutions (20% v/v) from

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95% ethanol and was used as the control solution. This dose of ethanol and volume of injection is consistent with previous reports using comparable conditioned place-preference paradigms with ethanol (Reid, Hunter, Beaman, & Hubbell, 1985; Bozarth, 1990).

<div align="center">Procedure</div>

Place Preference Conditioning

Subjects were tested using the procedure of Carboni et al. (1989) (see Table 1 for timeline). The test apparatus consisted of two Gemini shuttle-box avoidance systems (San Diego Instruments, San Diego, CA). Each shuttle-box was comprised of two 21 x 25 x 17 cm compartments separated by a vertically sliding door. The wire grid floor of one chamber in each shuttle-box was covered with a clear plastic plate which provided subjects with a unique cue by which to differentiate the chambers. Pilot data, collected using the procedures outlined for Experiment 1, revealed no evidence of an unconditioned preference for either floor surface. Both chambers of each shuttle-box were darkened throughout the place-preference portion of the experiment. The place-preference portion of the experiment was conducted over the course of 10 experimental sessions for each subject.

During the first two days of place-preference conditioning, the door was raised and subjects were allowed to roam freely throughout both compartments of the apparatus for 15 minutes per day. On the third day, subjects were given access to both compartments again and the time spent in each compartment was recorded. The purpose of this procedure was to determined the

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unconditioned preference of each rat for each of the two compartments. Preference for a given compartment was assumed if a subject occupied that compartment for a total of more than 450 seconds on day 3.

On each of the next five days (days 4-8, inclusive), subjects received a single injection of 0.8 mg/kg nicotine and a single injection of saline (SC) and were placed in either their preferred, or their non-preferred compartment. For half of the subjects, nicotine was paired with the preferred compartment whereas for the other half of the subjects, nicotine was paired with the non-preferred compartment. For nicotine-treated subjects, the compartment that was paired with nicotine is referred to as the "conditioned" compartment, whereas the compartment that was paired with saline is referred to as the "non-conditioned" compartment. For control subjects (i.e., those that received only saline during conditioning), the "conditioned" compartment was designated to match the nicotine-paired compartment of that subject's corresponding case. Similarly, the "non-conditioned" compartment was designated to match the saline-paired compartment of that subject's corresponding case (see Table 3 for a listing of case-control pairings). Subjects were exposed to each compartment for 30 minutes on each of these five days (days 4-8). Exposures to the conditioned and unconditioned chambers were spaced by at least four hours and were presented in a balanced order.

On the 9th day of the place-preference conditioning phase, the vertically-sliding door that separates the two compartments was lifted and the time spent in each compartment was recorded for 15 minutes. The time spent in the

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nicotine-paired compartment prior to the drug pairings and the time spent in the nicotine-paired compartment following the drug pairings were compared as a measure of place preference conditioning induced by nicotine. A saline control group, that received saline paired with both chambers, was included to allow between-subjects analyses of nicotine-induced conditioned place preference.

Ethanol Test

On the 10th day of the place-preference conditioning experiment, subjects received 1.0 g/kg ethanol, saline, or 1.0 g/kg ethanol administered with 0.8 mg/kg nicotine. This saline injection volume was adjusted to ensure that experimental and control subjects received injections of comparable volume. The time spent in each compartment following the ethanol or saline injections then was recorded for 15 minutes. The difference between the time spent in the nicotine-paired compartment on day 9 and the time spent in the nicotine-paired compartment on day 10 was taken as an index of ethanol-induced potentiation of nicotine's place-preference inducing effects. The group that received saline in both chambers was used for between-subjects comparisons of place preference on day 9. Immediately after the last place-preference test session on day 10, subjects were decapitated without anesthesia and the brains were removed and dissected for later assay of dopamine, l-DOPA, DOPAC, and HVA in nucleus accumbens.

Biochemical Assay

Biochemical measurement of dopamine, l-DOPA, DOPAC, and HVA levels in the nucleus accumbens was performed using High Performance Liquid

Chromatography (HPLC) according to published procedures (Shoami, Segal, & Jacobowitz, 1983; Zuddas, Corsini, Schinelli, Johannessen, Porzio, et al., 1989) and as described in Appendix A. It is important to note that Experiment 1 contains no *a priori* hypotheses regarding effects of nicotine and/or ethanol on levels of l-DOPA, DOPAC, or HVA. This reflects the design of Experiment 1 as initially proposed. Data for l-DOPA, DOPAC, and HVA were made accessible only after events forced a change in the biochemical analysis method. Data for l-DOPA, DOPAC, and HVA have been treated in the same manner as that used for dopamine in all statistical procedures.

<u>Statistical Analyses</u>

Repeated-measures ANOVA were used to examine effects of repeated nicotine treatments on place preference conditioning, latency to cross into or out of the conditioned chamber, and total number of crossings in: (1) subjects for which nicotine was paired with the "preferred" compartment, and (2) subjects for which nicotine was paired with the "non-preferred" compartment. Subjects that received saline only during conditioning were matched with subjects that received nicotine in a matched "case-control" design. This procedure ensured that every nicotine treated subject was paired with a comparable control subject that received saline in the same chamber (i.e., preferred or non-preferred). Between-subjects ANOVA were used to examine place conditioning, latency to cross, and total number of crossings in the nicotine treatment groups relative to the group that received saline only.

To examine effects of a single acute injection of ethanol on place preference induced by repeated injections of nicotine, repeated-measures ANOVA were used. One-way, between-subjects ANOVA, with Tukey HSD post-hoc tests, were used to determine differences between individual treatment groups at each time point. All statistical tests were two-tailed and used an alpha level of 0.05 or less to determine statistical significance. Outliers were eliminated from analyses pairwise and were defined as subjects having scores more than two standard deviations above or below the treatment group mean. Pairwise deletion refers to the elimination of outlying subjects from individual analyses rather than from the entire data set. This procedure helps to preserve degrees of freedom for analyses wherein subjects do not meet the criteria to be eliminated as outliers. The number of outliers ranged from 0-6 and were randomly distributed among the treatment groups.

Levels of dopamine, l-DOPA, DOPAC, and HVA in the nucleus accumbens are expressed as ng/mg protein. These data were analyzed using one-way ANOVA to examine effects of drug treatment on these biochemical measures. The ratios of dopamine/l-DOPA, dopamine/DOPAC, and dopamine/HVA also were analyzed using one-way ANOVA. Examining these ratios may provide indices of dopamine synthesis and metabolism related to enzymatic activity. Tukey's HSD comparisons were used to determine differences between individual treatment groups. Step-wise regression analyses were used to examine the relationship between levels of dopamine, l-DOPA, DOPAC, and HVA, and the behavioral effects of nicotine and ethanol

observed on testing days. Similarly, step-wise regression analyses were used to examine the relationship between the ratios of dopamine/l-DOPA, dopamine/DOPAC, and dopamine/HVA, and the behavioral effects of nicotine and ethanol observed on testing days. All tests were two-tailed and used an alpha level of 0.05 or less to determine significance.

Results

Behavioral Measures

Repeated-measures ANOVA were used to examine place preference conditioning by nicotine and to examine effects of nicotine and/or ethanol treatment on place preference conditioning. Repeated-measures ANOVA also were used to examine effects of place preference conditioning, and effects of nicotine and/or ethanol treatment, on the latency to cross between the two shuttle-box chambers, and on the total number of crossings recorded during the 15 minute test session. Result of these repeated-measures analyses are summarized in Table 4. Results of subsequent between-subjects analyses are summarized in Table 5.

Figures 3 and 4 present place preference in subjects that received nicotine or saline during the conditioning portion of the present experiment (days 4-8). Place preference was defined as the amount time (in seconds) that a subject spent in the "conditioned" chamber relative to the amount time that a subject spent in the "unconditioned" chamber during a given experimental session. A positive value indicates a preference for the conditioned chamber,

whereas a negative value indicates a preference for the unconditioned chamber.

Data for subjects that had the non-preferred chamber designated as the conditioned chamber are presented in Figure 3. Data for subjects that had the preferred chamber designated as the conditioned chamber are presented in Figure 4. Repeated-measures ANOVA indicate that effects of nicotine to induce a place preference were not statistically significant. Similarly, effects of acute nicotine and/or ethanol treatment on place preference conditioning by nicotine were not statistically significant.

Figures 5 and 6 present the latency to cross into the saline-paired chamber (among subjects that began the session in the drug-paired chamber) or the latency to cross into the drug-paired chamber (among subjects that began the session in the saline-paired chamber), respectively. Latencies to enter and/or exit the nicotine conditioned chamber provide an index of reward conditioning induced by nicotine. Shorter latencies to cross into the nicotine-paired chamber (or longer latencies to cross *out of* the nicotine-paired chamber) indicates a preference for the nicotine-paired side. Longer latencies to cross into the nicotine-paired chamber (or shorter latencies to cross *out of* the nicotine-paired chamber) indicates a preference for the saline-paired side.

Among nicotine-treated subjects that began the test sessions in the saline-paired chamber (Figure 6b), there was a statistically significant effect of time on the latency of subjects to cross between chambers [$F_{(2,36)}=5.93$, $p<.05$]. Specifically, all subjects had shorter latencies on the ethanol test day

than they had on the previous test days regardless of acute drug treatment. Subsequent ANOVA, examining effects on each of the three test days individually, failed to reveal statistically significant differences between treatment groups.

Among nicotine-treated subjects that began the test session in the nicotine-paired chamber (Figure 5b), there was a significant time by day treatment group interaction on the latency of subjects to cross between chambers [$F(4,38)=2.598$, $p<.05$]. Day 10 was the ethanol test day and was the only day on which subjects received ethanol. On day 10, the saline and ethanol groups had slightly shorter latencies to cross on the ethanol test day than they did on the place preference test day, whereas the nicotine plus ethanol group had slightly longer latencies to cross on the ethanol test day than they had on the place preference test day. The fact that the ethanol plus nicotine groups had longer latencies to cross on the ethanol test day than they had on the previous test days suggests a possible interactive effect of nicotine and ethanol that may be relevant to nicotine reward. However, because there were no significant effects of nicotine to condition place preference generally, effects of nicotine and ethanol observed on the ethanol test day should be regarded tentatively.

Figures 7 and 8 present the total number of times that subjects crossed the midline between the conditioned and unconditioned chambers during the 15 minute test sessions. The total number of crossings provides an index of the

subjects' general activity level and perhaps provides an index of stimulating and/or depressing drug effects.

Figure 7 presents the number of crossings for subjects that were conditioned in the non-preferred chamber and Figure 8 presents the number of crossings for subjects that were conditioned in the preferred chamber. Among control subjects that were conditioned using saline in the non-preferred chamber (Figure 7a), there was a significant main effect of time [$F_{(2,40)}$= 23.607, $p<.05$] and a significant time by day 10 treatment group interaction [$F_{(4,40)}$= 3.974, $p<.05$]. Day 10 was the ethanol test day and was the only day on which subjects received ethanol. Data collected on day 10 reflect the effects of the place-preference conditioning procedure (days 4-8, inclusive) on subsequent responses to ethanol. This main effect of time indicates that the number of crossings generally differed across test days. On the ethanol test day (day 10), subjects that received ethanol alone exhibited fewer crossings than did subjects that received only saline ($p<.05$). This effect of ethanol to inhibit crossings was not evident in subjects that also received nicotine, suggesting an effect of nicotine to offset some effects of ethanol. Among subjects that were conditioned using nicotine in the non-preferred chamber (Figure 7b), there was a significant effect of time [$F_{(2,32)}=6.22$, $p<.05$] and a significant time by day 10 treatment group interaction [$F_{(4,32)}=4.90$, $p<.05$]. This main effect of time indicates that the number of crossings generally differed across test days. On day 10, subjects that received ethanol alone exhibited significantly fewer crossings on day 10 than did the group that received only

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saline ($p<.05$).   This effect of ethanol to inhibit crossings was not evident in subjects that also received nicotine, again suggesting that nicotine can offset some of the effects of ethanol. The fact that ethanol alone had similar effects on the number of crossings regardless of whether subjects were previously conditioned with nicotine or with saline suggests that prior exposure to nicotine does not alter effects of ethanol in the absence of acute nicotine.

Among control subjects that were conditioned using saline in the preferred chamber (Figure 8a), there was a significant effect of time [$F_{(2,36)}=17.72$, $p<.05$] and a time by day 10 treatment group interaction that approached significance [$F_{(4,36)}=2.55$, $p=.056$]. This main effect of time indicates that the number of crossings generally differed across test days. On day 10, subjects that received ethanol alone exhibited fewer crossings than did subjects that received only saline ($p<.05$). This effect of ethanol to inhibit crossings was not evident in subjects that also received nicotine, suggesting an effect of nicotine to offset some effects of ethanol. Among subjects that were conditioned using nicotine in their preferred chamber (Figure 8b), there was a significant effect of time [$F_{(2,40)}=10.48$, $p<.05$] but no time by drug treatment interaction. This main effect of time indicates that the number of crossings generally differed across test days. On day 10, subjects that received ethanol alone exhibited significantly fewer crossings than did subjects that received ethanol with nicotine ($p<.05$). This result suggests that ethanol can depress locomotor activity and that nicotine can offset this ethanol-induced locomotor depression.   The fact that ethanol alone had similar effects on the number of

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crossings regardless of whether subjects were previously conditioned with nicotine or whether subjects were previously conditioned with saline suggests that prior exposure to nicotine does not alter effects of ethanol in the absence of acute nicotine.

<div align="center">Biochemical Measures</div>

One-way ANOVA was used to examine effects of drug treatment, administered on the ethanol test day, on levels of dopamine, I-DOPA, DOPAC, and HVA in nucleus accumbens. Similar analyses were used to examine effects of drug treatment on the ratio of dopamine/I-DOPA, the ratio of dopamine/DOPAC, and the ratio of dopamine/HVA. The purpose of these analyses was to determine effects of ethanol alone, and in combination with nicotine, on dopamine synthesis and metabolism. Increases in the ratio of dopamine/I-DOPA indicate increases in the rate of dopamine synthesis. Increases in the ratio of dopamine/DOPAC, and in the ratio of dopamine/HVA, indicate decreases in the rate of dopamine metabolism. Step-wise multiple regression analyses were used to examine the relationship between the biochemical variables and the behavioral variables measured on the day of sacrifice. Results of all ANOVA are presented in Table 8. Correlations coefficients, describing the relationship between behavioral and biochemical variables, are presented in Table 6.

Figures 9a and 9b present effects of drug treatment on the ratio of dopamine/DOPAC in nucleus accumbens of subjects that received saline or nicotine, respectively, during conditioning. Figures 10a and 10b present the

same data with dopamine and DOPAC represented separately. ANOVA revealed a significant effect of drug treatment to reduce the dopamine/DOPAC ratio in subjects that received nicotine during conditioning [$F_{(5,94)}=3.48$, $p<.05$]. There was no such effect in subjects that received saline during conditioning. Tukey's *a posteriori* comparisons indicate that subjects that received nicotine and ethanol together (and that had received nicotine during conditioning) had lower dopamine/DOPAC ratio than did subjects that received ethanol alone ($p<.05$). This finding suggests that repeated treatments with nicotine can alter acute responses to nicotine and ethanol. More specifically, this finding suggests that repeated injections of nicotine can lead to increases in the rate of dopamine turnover immediately following acute treatment with nicotine and ethanol. There were no statistically significant effects of drug treatment on any of the other central biochemical measures. In addition, multiple regression analysis did not reveal any significant relationships between behavioral and biochemical variables.

<u>Confirmation of Major Hypotheses</u>

Hypothesis 1, that subjects would exhibit a preference for an environment that was paired with nicotine relative to an environment that was paired with saline, was not confirmed. There was no relationship between nicotine administration and place preference conditioning in the present experiment.

Hypothesis 2, that administration of ethanol would potentiate conditioned place preference for nicotine, was not confirmed. Because there was no place

preference associated with nicotine conditioning, there could not have been an effect of ethanol to potentiate this place preference.

Discussion

The present experiment had two main purposes. The first purpose was to examine effects of repeated nicotine injections to condition place preference in rats. The second purpose was to examine effects of acute ethanol injections to modify the conditioned place preference induced by nicotine. Contrary to predictions, repeated administration of nicotine did not condition place preference in this animal model (Figures 3-4). Further, acute injection of ethanol did not significantly alter subjects' place-preference. There are several possible explanations for these results. First, it is possible that the failure of nicotine to condition a place preference reflects a general absence of reinforcement from nicotine. Although this explanation is consistent with present data, it is not consistent with previous reports using nicotine self-administration in rats (Corrigall & Coen, 1991, 1994; Corrigall, et al. 1992, 1994) or with a preponderance of experimental evidence regarding nicotine addiction in humans (USDHHS, 1988). A second possible explanation for present results lies in the methodologic differences between experiments that report conditioned place preference with nicotine (Fudala, et al., 1985; Fudala & Iwamoto, 1986; Acquas, et al., 1989; Carboni, et al., 1989) and experiments that report a failure to condition place preference with nicotine (Clarke & Fibiger, 1987; Jorenby, Steinpreis, Sherman, & Baker, 1990). In the former experiments, subjects received 3-4 exposures to nicotine (0.8 mg/kg; SC) prior

to conditioned place preference testing. In the latter experiments, subjects received 4-5 exposures to nicotine prior conditioned place preference testing. Clarke & Fibiger (1987) for example, used an eight day (4 nicotine exposure) conditioning procedure and reported no place preference conditioning by 0.8 mg/kg nicotine (SC). Jorenby, et al. (1990) used a 10-day conditioning procedure (with 5 days of nicotine exposure) and reported conditioned place aversion by 0.8 mg/kg nicotine (SC). Presently, it was presumed that the failure of Clarke & Fibiger (1987) and of Jorenby, et al. (1990) to condition place preference with nicotine resulted from the fact that nicotine exposures were separated by days on which subjects did not receive nicotine. It was believed that this sporadic administration procedure may have weakened the subsequent association to be formed between the effects of nicotine and the nicotine-paired environment, thereby inhibiting the formation of conditioned place preference. In the present experiment, subjects received five nicotine-environment pairings over five days. The purpose of exposing subjects to five nicotine-environment pairings over five days was to strengthen the subsequent association to be formed between the effects of nicotine and the nicotine-paired environment by eliminating the days on which subjects did not receive nicotine paired with the appropriate environment. Like previous experiments that used more than 3 nicotine-environment pairings, however, there was no evidence of conditioned place-preference in the present experiment. This result suggests that the strength of nicotine-induced place preference conditioning may vary as a function of the number of nicotine-environment pairings and not as a function of

the timing of the nicotine-environment pairings as originally supposed. More specifically, it seems that the strength of nicotine-induced place preference conditioning may follow a curvilinear function of drug-environment exposures, with low numbers of exposures conditioning preference and higher numbers of exposures either failing to condition preference or actually conditioning aversion to nicotine. Although this interpretation is consistent with present findings, as well as those of Clarke & Fibiger (1987) and Jorenby, et al. (1990), it is contrary to the nature of other forms of conditioning which follow an ascending, asymptotic function that does not weaken with additional CS-UCS pairings (Rescorla & Wagner, 1972).

A second important finding of the present experiment is that acute nicotine treatment offset ethanol's effects on locomotor exploratory behavior as indicated by the number of crossings between chambers (Figures 7-8). When ethanol was administered to subjects that did not also receive nicotine, the subjects had significantly lower numbers of crosses than did subjects that received saline. When ethanol was administered with nicotine, however, these effects of ethanol were reduced. That is, nicotine treatment offset the locomotor inhibiting effects of ethanol to a level that did not differ from control. This effect existed regardless of whether subjects had received nicotine or saline during conditioning. These effects of nicotine and ethanol are consistent with previous reports that nicotine and ethanol can interact to alter psychological and motor performance (Lyon, et al., 1975; Schaefer & Michael, 1992). In addition, these effects are consistent with the suggestion that nicotine may offset some of the

negative effects of ethanol and that these effects of nicotine may be responsible for the positive association between cigarette smoking and alcohol consumption (Shiffman, et al., 1994) In Experiment 2, these interactive effects of nicotine and ethanol are examined using the acoustic startle response paradigm as a measure of psychomotor performance.

Biochemically, the present experiment revealed an effect of concurrent nicotine and ethanol treatment to reduce the ratio of dopamine/DOPAC in nucleus accumbens (Figure 9). This effect was evident in all subjects, but was statistically significant only in those that had received nicotine during conditioning. This result suggests that nicotine and alcohol can combine to increase the rate of dopamine turnover and that this effect may be dependant on prior exposure to nicotine. This result is consistent with the findings of Johnson, Blomqvist, Engel, & Söderpalm (1995) who reported an effect of repeated nicotine injections to potentiate ethanol-induced increases in dopamine turnover in mouse brain. Because dopaminergic activity in nucleus accumbens is known to mediate nicotine reward, interactive effects of nicotine and ethanol in this region may suggest a mechanism that may underlie the tendency of some individuals to smoke more when they drink.

Because the present experiment did not reveal significant changes in levels of dopamine or in levels of DOPAC, independently, it is difficult to determine whether effects of nicotine and ethanol to reduce the ratio of dopamine/DOPAC result from an increase in the conversion of dopamine to DOPAC, a decrease in the synthesis of dopamine (independent of its

conversion to DOPAC), or neither. Rommelspacher, et al. (1994) reported that levels of MAO-B, the intracellular enzyme responsible for the conversion of dopamine to DOPAC, are reduced in the platelets of alcoholics during chronic intoxication and in the platelets of normal controls during acute intoxication. Because MAO-B is the enzyme responsible for the conversion of dopamine to DOPAC, and because ethanol appears to decrease MAO-B activity, it seems unlikely that effects of nicotine and ethanol to reduce the dopamine/DOPAC ratio result from effects of ethanol to independently increase the conversion of dopamine to DOPAC. Because nicotine has been reported to have similar effects to reduce MAO-B (Fowler, Volkow, Wang, Pappas, Logan, et al., 1996), it seems unlikely that effects of nicotine and ethanol to reduce the dopamine/DOPAC ratio result from effects of nicotine to independently increase the conversion of dopamine to DOPAC.

It seems equally unlikely that nicotine and/or ethanol reduce the dopamine/DOPAC ratio by reducing the rate of dopamine synthesis. In fact, recent reports suggest that nicotine and ethanol can each increase the rate of dopamine synthesis by increasing the activity of the rate-limiting, dopamine-synthesizing enzyme, tyrosine hydroxylase (Naquira, Zunnino, Arqueros, & Viveros, 1978; Smith, Mitchell, Joseph, 1991). Because increases in dopamine synthesis would tend to increase the ratio of dopamine/DOPAC, it seems unlikely that effects of nicotine and ethanol on dopamine synthesis underlie effects reported presently.

A third possible explanation for effects of nicotine and ethanol to decrease the dopamine/DOPAC ratio is that nicotine and ethanol have effects to increase the conversion of dopamine to norepinephrine intracellularly, and that these effects result in a reduction of available dopamine and a reduction in the dopamine/DOPAC ratio. It is known, for example, that nicotine can increase production of dopamine β-hydroxylase, the enzyme that converts dopamine to norepinephrine inside the nerve terminal (Bhargava & Sabban, 1995; Hofle, Weiler, Fischer-Colbrie, Humpel, Laqsolp, et al., 1991). To the extent that the repeated injections of nicotine administered presently resulted in an increase in the rate of conversion of dopamine to norepinephrine, it is possible that the resulting reductions in dopamine contributed to the reduced dopamine/DOPAC ratio observed presently. It is important to note, however, that in the present experiment, there were no significant effects of nicotine and ethanol to reduce dopamine levels *per se*--only to reduce the ratio of dopamine/DOPAC. Future experiments, examining specific enzyme activities and transmitter levels in response to separate and combined treatment with nicotine and/or ethanol may help to clarify the mechanisms that underlie effects of nicotine and ethanol on the ratio of dopamine/DOPAC.

EXPERIMENT 2: EFFECTS OF ACUTE ETHANOL AND ACUTE NICOTINE ON THE AMPLITUDE OF THE ACOUSTIC STARTLE RESPONSE AND ON PRE-PULSE INHIBITION IN MALE RATS

Overview

The purpose of Experiment 2 was to examine the separate and combined effects of nicotine and ethanol on the amplitude of the acoustic startle response (ASR) and on pre-pulse inhibition of the acoustic startle response (PPI) in rats. These behavioral measures have been used to study processes that may underlie sensory-gating (Swerdlow, Braff, Geyer, & Koob, 1986) and possibly attention (Acri, Grunberg, & Morse, 1991; Acri, et al., 1994; Grunberg, et al., 1994). Because nicotine's effects on cognitive and behavioral processes may play a role to reinforce smoking behavior (Grunberg, et al., 1983; USDHHS, 1988), it is relevant to understand whether nicotine's effects on a behavioral measure of sensory-gating may be altered by ethanol.

In Experiment 2, effects of nicotine and ethanol on ASR and on PPI were assessed in rats using a 4-station acoustic startle test system (Coulbourn Instruments, Allentown, PA). Baseline testing consisted of a single test session in which subjects received no injections and a second session in which subjects received two injections of saline. During the third session, subjects were tested following treatment with one of the twelve dosing regimens outlined in Table 7. Each test session included eight presentations of each stimulus intensity (112 and 122 dB) both with and without a 68 dB pre-pulse. The order of presentation was randomized within blocks to ensure that each stimulus type was presented

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within seven trials of its last presentation and that none of the stimuli occurred more than once in sequence. Inter-trial intervals ranged randomly from 10 - 30 seconds. Effects of nicotine and ethanol were assessed alone and in combination as they may relate to the propensity of smokers to smoke more when they drink.

Seven days after testing, subjects received injections of nicotine and/or ethanol and were sacrificed by decapitation. Striatum was removed from whole brain and was frozen at -80° C until later assay for dopamine, l-DOPA, DOPAC, and HVA. Biochemical analyses were performed using High Performance Liquid Chromatography (HPLC) according to published procedures (Shoami et al., 1983; Zuddas, et al., 1989) and as described in Appendix A.

Methods

Subjects and Housing

Subjects were 96 experimentally-naive, male Sprague-Dawley rats. Subjects were roughly 8 weeks old and weighed 225 g at the beginning of Experiment 2. The use of non-human subjects permits pharmacologic manipulations and experimental procedures that are not ethically feasible in human subjects. The use of Sprague-Dawley rats, specifically, is reflective of the published literature and allows comparisons to be made between the results of the present experiment and results of previous experiments (Acri, et al., 1991; Acri, et al., 1994; Popke, et al., 1994). Age of subjects also is consistent with published reports to allow comparison between results of the present

experiment and results of previous experiments. The sample of 96 subjects included 16 subjects in each between-subjects comparison in Experiment 2 (8 subjects in each experimental group). This sample size affords an 80% probability of detecting significant experimental effects given an alpha level of 0.05 and an experimental effect size of 0.7 (Cohen & Cohen, 1988). This effect size was determined based on published reports using similar behavioral measures (Acri, et al., 1994; Grunberg, et al., 1994; Popke & Grunberg, 1994).

Animals were housed individually in 35.6 cm x 15.2 cm x 20.3 cm cages with absorbent Pine-Dri, wood-chip bedding. Animals were maintained under a 12 h light/dark cycle (lights on at 0700) at approximately 23 degrees C and 50% relative humidity. Water and laboratory chow (Agway Prolab 3200) were available continuously. These housing conditions were consistent with previous reports using similar behavioral measures (Acri, et al., 1994; Grunberg, et al., 1994; Popke & Grunberg, 1994).

Nicotine Administration

During acoustic startle testing, subjects received injections of either saline, 0.01 mg/kg nicotine, or 0.5 mg/kg nicotine administered subcutaneously (SC) in volumes ranging from 0.2 to 0.35 ml. Physiological saline (0.9% NaCl) was used to prepare the nicotine solutions from nicotine dihydrochloride and was used as the control solution. These dosages were computed as base and were consistent with previous reports using SC nicotine in this paradigm (Acri, et al., 1994; Grunberg, et al., 1994; Popke & Grunberg, 1994). Volumes of the

injections also were consistent with previous reports (Acri, et al., 1994; Grunberg, et al., 1994; Popke & Grunberg, 1994).

Ethanol Administration

Subjects received injections of 0.5 g/kg, 1.0 g/kg, or 2.0 g/kg ethanol administered intraperitoneally (IP) in volumes ranging from 1.5 to 3.0 ml. Physiological saline (0.9% NaCl) was used to prepare solutions (20% v/v) from 95% ethanol and was used as the control solution. Because little is known regarding effects of ethanol on ASR or PPI, multiple doses of ethanol were used. These dosages and injection volumes were consistent with previous studies examining behavioral effects of acute ethanol in rats (LeBlanc, Gibbins, & Kalant, 1975; Pohorecky, Cagan, Brick, & Jaffe, 1976; Brick, Pohorecky, Faulkner, & Adams, 1984; Schaefer & Michael, 1992; Franklin & Abbott, 1993; Heidbreder & Philippe, 1993).

Startle and Pre-Pulse Testing

Acoustic startle was tested using a four-station acoustic startle system (Coulbourn Instruments, Allentown, PA) based on the procedures of Acri, Grunberg, and Morse (1991). Specifically, animals were enclosed in 8 x 8 x 16 cm open air cages that restricted locomotion but did not restrain the animal. Cages were placed on one of four platforms in a sound attenuating test chamber (e.g., 4 subjects were tested simultaneously). Although it has been reported that some rats emit ultrasounds during startle testing (Miczek, Vivian, Tornatsky, Farrell, & Sapperstein, 1993), there is no evidence to support any speculation that these ultrasounds can influence acoustic startle responding

within subjects. Background noise within the sound-attenuating startle chamber was produced by a ventilating fan measured at 56 dB. Startle-eliciting acoustic stimuli consisted of 20 ms noise bursts of 112 dB SPL or 122 dB SPL. Each startle-eliciting acoustic stimulus had a 2 ms rise and decay time such that onset and offset are abrupt, a primary criterion for startle. Pre-pulse stimuli consisted of a 20 ms, 1 KHz pure tone of 68 dB SPL (12 dB above background). The intensity of this pre-pulse is comparable to those used by Curzon, Kim, & Decker (1994). The onset of the pre-pulse stimuli preceded the onset of the startle-eliciting stimuli by 100 msec. Trials with no stimuli and trials with only pre-pulse also were presented. Each subject's movement in response to each stimulus was measured as voltage change by a strain gauge and was converted to grams of body weight change following analog to digital conversion. Responses were recorded by an interfaced microcomputer as the maximum response occurring within 200 msec of the onset of the startle-eliciting stimuli. A single test session included eight presentations of each stimulus intensity both with and without pre-pulse. The order of presentation was randomized within blocks to ensure that each stimulus type was presented within seven trials of its last presentation and that none of the stimuli occurred more than once in sequence. Inter-trial intervals ranged randomly from 10 - 30 seconds.

Procedure

Behavioral Testing

Baseline testing consisted of one test session with no treatment and a second test session in which subjects received two injections of physiologic saline (0.9% NaCl). The purpose of the first baseline session was to acclimate subjects to the startle procedure and to reduce the likelihood that the stress of a novel environment contributed to experimental effects observed during treatment. During the second baseline session, each animal received two injections of physiologic saline. The purpose of this second baseline session was to familiarize the animals with the injection procedure to further minimize stress effects on PPI during subsequent drug treatments. Data from these baseline sessions were not included in subsequent statistical analyses. Test sessions for each animal were separated by at least four days to minimize effects of habituation on PPI (Thompson & Spencer, 1966).

Four days after the second baseline session, subjects were treated once using one of the following dosing regimens: (1) saline administered following saline; (2) 0.01 mg/kg nicotine administered following saline; (3) 0.5 mg/kg nicotine administered following saline; (4) saline administered following 0.5 g/kg ethanol; (5) 0.01 mg/kg nicotine administered following 0.5 g/kg ethanol; (6) 0.5 mg/kg nicotine administered following 0.5 g/kg ethanol; (7) saline administered following 1.0 g/kg ethanol; (8) 0.01 mg/kg nicotine administered following 1.0 g/kg ethanol; (9) 0.5 mg/kg nicotine administered following 1.0 g/kg ethanol; (10) saline administered following 2.0 g/kg ethanol; (11) 0.01 mg/kg nicotine

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administered following 2.0 g/kg ethanol; or (12) 0.5 mg/kg nicotine administered following 2.0 g/kg ethanol. The second injection (saline or nicotine) was administered 10 minutes after the first injection (saline or ethanol). ASR and PPI were evaluated 15 minutes after the second injection. The time between the first injection and the end of acoustic startle testing was approximately 40 minutes in all treatment conditions. All manipulations were conducted during the subjects active (dark) cycle to maintain consistency with respect to the animals' circadian activity.

Seven days after the last ASR/PPI test session, subjects received a second treatment using the same dosing regimens used on the ASR/PPI test day. Because administration of 2.0 g/kg ETOH during ASR testing resulted in the death of nine (out of 24) subjects and in visible injury (severe weight loss, lethargy, muscular atrophy) to the remaining fifteen, all 2.0 g/kg ETOH subjects were eliminated from the experiment prior to sacrifice. Therefore, no biochemical data were collected from the 2.0 g/kg ETOH subjects. Fifteen minutes after the second injection, subjects were decapitated without anesthesia and the brains were removed and dissected for later assay of l-DOPA, dopamine, DOPAC, and HVA levels in the striatum. It is important to note that the 0.5 g/kg ETOH groups were not part of the experiment as initially proposed. These subjects were added only after the 2.0 g/kg ETOH subjects became ill and were eliminated from the experiment. The addition of the 0.5 g/kg ETOH groups helped to maintain the integrity of the original proposal by

ensuring that effects of two different doses of ETOH could be included in all biochemical analyses.

<u>Biochemical Testing</u>

Biochemical measurement of l-DOPA, dopamine, DOPAC, and HVA levels in the striatum was performed using High Performance Liquid Chromatography (HPLC) according to published procedures (Shoami, et al., 1983; Zuddas, et al., 1989) and as described in Appendix A. It is important to note that Experiment 2 contains no *a priori* hypotheses regarding effects of nicotine and/or ethanol on l-DOPA, DOPAC, or HVA. This reflects the design of Experiment 2 as initially proposed. Data for l-DOPA, DOPAC, and HVA were made accessible only after events forced a change in the biochemical analysis method. Data for l-DOPA, DOPAC, and HVA have been treated in the same manner as that used for dopamine in all statistical procedures.

<u>Treatment of Data and Statistical Analyses</u>

Data analytic strategies used presently are consistent with previous reports (Acri, et al., 1991; Acri, 1994; Acri, et al., 1994; Grunberg, et al., 1994; Popke, et al., 1994; Popke, et al., 1994; Popke, et al., 1995). Specifically, startle amplitudes were determined for each animal by subtracting the response to the no-stimulus control trials from the average peak response recorded during each of the other trial types. Measurement of responses during no-stimulus control trials provides a measure of subjects' body weight. Subtracting this value from the responses measured during each of the other trial types: (1) helps to control for differences in body weight; (2) helps to control for random

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movement of subjects on the acoustic startle platforms; and (3) provides a means for assessing the functioning of the acoustic startle platforms. The amount of pre-pulse inhibition was determined by subtracting the response to the pre-pulse trials from the response to the trials in which the same stimulus was presented without pre-pulse. The amount of pre-pulse inhibition was divided by the response amplitude from trials using similar stimuli without pre-pulse to determine the percentage of the response inhibited. Data were analyzed using two-way ANOVA with the two injections entered as between-subjects factors. Tukey's HSD tests were used to determine differences between dose groups of nicotine and ethanol. All tests were two-tailed and use an alpha level of 0.05 or less to determine significance. Outliers were eliminated from analyses pairwise and were defined as subjects having scores more than two standard deviations above or below the group mean. Pairwise deletion refers to the elimination of outlying subjects from individual analyses rather than from the entire data set. This procedure helps to preserve degrees of freedom for analyses wherein subjects do not meet the criteria to be eliminated as outliers. The number of outliers ranged from 0-4 and were randomly distributed among the treatment groups. Table 8 provides a summary of ANOVA results. Correlation coefficients, describing the relationship between behavioral and biochemical variables, are presented in Table 9.

Levels of l-DOPA, dopamine, DOPAC, and HVA in the striatum are expressed as ng/mg protein. Because tissue samples from several subjects were lost during handling, remaining samples from members of the same

treatment group were divided and were substituted for missing subjects during biochemical analyses. These data were analyzed using two-way ANOVA with nicotine dose and ethanol dose entered as between-subjects factors. Correlation analyses were used to examine the relationship between ASR and PPI observed on testing days and levels of dopamine, l-DOPA, DOPAC, and HVA measured following nicotine and/or ethanol treatment on the day of tissue collection. Similar analyses were used to examine effects of drug treatment on the ratios of dopamine/l-DOPA , dopamine/DOPAC, and dopamine/HVA, and to examine the relationships between these ratios and ASR and PPI measured on the behavioral testing day. All tests were two-tailed and used an alpha level of 0.05 or less to determine significance.

Results

Behavioral Measures

The acoustic startle response (ASR) and pre-pulse inhibition of the acoustic startle response (PPI) provide sensitive indices of reactivity that have been interpreted to reflect processes that underlie sensory-gating (Swerdlow, et al., 1986) and possibly attention (Acri, et al., 1991; Acri, et al., 1994; Grunberg, et al., 1994). In the present experiment, these measures were used to examine interactions of nicotine with ethanol as they may act to reinforce smoking behavior following alcohol consumption.

ASR and PPI data were analyzed by two-way ANOVA with the first (ethanol) and second (nicotine) injections as between subjects factors. Figure 11 presents ASR (Figure 11a), PPI (Figure 11b), and percent PPI (Figure 11c)

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measured when the 112 dB stimulus was presented. For ASR, ANOVA revealed a significant main effect of ethanol [$F_{(3,79)}=9.62$, $p<.05$], an effect of nicotine that approached significance [$F_{(2,79)}=2.41$, $p=.097$], and a significant injection 1 by injection 2 interaction [$F_{(6,79)}=2.85$, $p<.05$] on ASR. These results illustrate a curvilinear effect of ethanol to affect ASR with the lowest dose of ethanol increasing ASR and the two higher doses reducing ASR relative to controls. The startle-increasing effects of ethanol were reduced in the presence of nicotine. Tukey's *a posteriori* comparisons indicated that subjects treated with 0.5 g/kg ethanol and saline had greater startle amplitudes than all other subjects except those that received saline alone.

For PPI, there was a significant main effect of ethanol [$F_{(3,78)}=4.37$, $p<.05$] with subjects that received 0.5 g/kg ethanol with saline having greater PPI than subjects that received either 2.0 g/kg ethanol with saline, 1.0 g/kg ethanol with 0.5 mg/kg nicotine, or 2.0 g/kg ethanol with 0.5 mg/kg nicotine. Effects of drug treatment on percent PPI were not statistically significant.

Figure 12 presents ASR (Figure 12a), PPI (Figure 12b) and percent PPI (Figure 12c) measured when the 122 dB stimulus was used. ANOVA revealed a significant main effect of ethanol [$F_{(3,81)}=9.382$, $p<.05$], a significant main effect of nicotine [$F_{(3,81)}=3.71$, $p<.05$], and a significant ethanol by nicotine interaction [$F_{(6,81)}=3.51$, $p<.05$] on ASR. Tukey's *a posteriori* comparisons indicated that the groups that received 2.0 g/kg ethanol with saline or with 0.5 mg/kg nicotine had lower ASR amplitudes than did subjects that received only saline. Additionally, the 0.5 g/kg ethanol + saline group had greater ASR

amplitudes than did any other group except those which received either 0.5

mg/kg nicotine with saline or saline only. Finally, the group that received 2.0

g/kg ethanol with 0.5 mg/kg nicotine had lower ASR amplitudes than did those

which received saline with 0.5 mg/kg nicotine. The fact that the impairing

effects of a 1.0 g/kg dose of ethanol were offset by a high dose of nicotine (0.5

mg/kg) suggest that nicotine can attenuate some of the impairing effects of

ethanol on ASR, and possibly on sensory-motor processing.

For PPI, there was a significant effect of ethanol [$F(3,81)=3.324$, $p<.05$]

but no effect of nicotine and no nicotine by ethanol interactions. Individual dose

groups also did not differ. Effects of drug treatment on percent PPI were not

statistically significant.

<u>Biochemical Measures</u>

Effects of nicotine and ethanol on levels of l-DOPA, dopamine, DOPAC,

and HVA in striatum were examined using two-way ANOVA with nicotine and

ethanol entered as between-subjects factors. The ratio of l-DOPA/Dopamine,

Dopamine/DOPAC, and Dopamine/HVA also were analyzed using two-way

ANOVA. The purpose of these analyses was to examine effects of drug

treatment on dopamine synthesis and turnover. In addition, the relative

contributions of each biochemical variable to the variance in ASR and PPI was

assessed using step-wise multiple regression analyses. The results of two-way

ANOVA are presented in Table 8. Correlation coefficients, describing the

relationship between behavioral and biochemical variables, are presented in

Table 9.

Figure 13 presents effects of nicotine and ethanol on levels of dopamine, l-DOPA, DOPAC, and HVA in striatum. With respect to dopamine levels in striatum (Figure 13a), ANOVA revealed a significant main effect of ethanol treatment [$F_{(2,61)}=16.15$, $p<.001$] and a significant interaction of ethanol with nicotine [$F_{(4,61)}=3.686$, $p<.01$]. Tukey's *a posteriori* comparisons indicate that subjects that received 0.5 g/kg ethanol with saline or with 0.01 mg/kg nicotine had lower dopamine levels in striatum than did subjects which received only saline. Additionally, subjects that received 0.5 mg/kg nicotine with saline had lower dopamine levels in striatum than did subjects that received only saline ($p<.05$). These results suggest an effect of nicotine (and possibly low-dose ethanol) to deplete dopamine levels in striatum. Because none of the groups that received a high dose of ethanol differed significantly from saline (regardless of nicotine treatment), the present results also may suggest an effect of high-dose ethanol to offset treatment-induced dopamine depletions. This finding provides evidence that ethanol may help to increase dopamine availability.

With respect to levels of l-DOPA in striatum (Figure 13b), two-way ANOVA revealed significant main effects of ethanol [$F_{(2,61)}=3.59$, $p<.05$] and nicotine [$F_{(2,61)}=6.14$, $p<.01$], as well as a significant ethanol x nicotine interaction [$F_{(4,61)}=4.80$, $p<.01$]. Tukey's *a posteriori* comparisons indicate that subjects that received 0.5 mg/kg nicotine without ethanol had significantly lower levels of l-DOPA in striatum than did subjects that received only saline ($p<.05$). Because none of the groups that had received ethanol had significant

reductions in l-DOPA relative to saline, the results suggest an effect of ethanol to offset effects of nicotine to reduce levels of l-DOPA in striatum.

With respect to levels of DOPAC in striatum (Figure 13c), two-way ANOVA revealed a significant main effect of ethanol [$F_{(2,61)}=8.06$, $p<.001$] and a significant interaction of ethanol with nicotine [$F_{(4,61)}=12.166$, $p<.001$]. Tukey's *a posteriori* comparisons indicate that subjects that received either dose of ethanol with saline, 0.5 g/kg ethanol with 0.01 mg/kg nicotine, or 0.5 mg/kg nicotine with saline had lower levels of DOPAC in striatum than did subjects that received saline only ($p<.05$).

With respect to levels of HVA in striatum (Figure 13d), two-way ANOVA revealed a significant main effect of ethanol [$F_{(2,61)}=3.563$, $p<.05$], a significant main effect of nicotine [$F_{(2,61)}=4.30$, $p<.05$], and a significant nicotine x ethanol interaction [$F_{(4,61)}=8.07$, $p<.001$]. Tukey's *a posteriori* comparisons indicate that subjects that received 0.5 mg/kg nicotine with saline had lower levels of HVA in striatum than did subjects that received only saline ($p<.05$). Because none of the groups that had received ethanol had significant reductions in HVA relative to saline, the results suggest an effect of ethanol to offset effects of nicotine to reduce levels of HVA in striatum.

To assess the relative contributions of dopamine, l-DOPA, DOPAC, and HVA levels to the measured variance in ASR and PPI, step-wise multiple regression analysis was used. HVA levels in striatum contribute significantly to the variance in ASR when the 112 dB stimulus was used [$R^2=.14$, $F(1,69)=10.91$, $p<.01$] and when the 122 dB stimulus was used [$R^2=.11$,

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F(1,69)=8.63, p<.01]. These results indicate that a significant inverse relationship exists between acoustic startle response amplitudes and levels of HVA in striatum. A similar inverse relationship was revealed for PPI in that levels of HVA contributed significantly to the variance in PPI when the 112 dB stimulus was used (R²=.11, F(1,69)=8.21, p<.01]. As was the case for ASR, this result suggests that a significant inverse relationship exists between pre-pulse inhibition and levels of HVA in striatum.

Figure 14 presents effects of nicotine and ethanol on the ratio of dopamine/l-DOPA, the ratio of dopamine/DOPAC, and the ratio of dopamine/HVA. Examination of these relationships may help to clarify effects of nicotine and alcohol to increase dopamine synthesis and to decrease dopamine metabolism. Specifically, increases in the ratio of dopamine/l-DOPA indicate an increase in the rate of dopamine synthesis. Increases in the ratio of dopamine/DOPAC and in the ratio of dopamine/HVA indicate decreases in the rate of dopamine metabolism.

With respect to the ratio of dopamine/l-DOPA (Figure 14a), two-way ANOVA revealed a significant main effect of nicotine [F(2,61)=4.16, p<.05] and a significant interaction of nicotine with ethanol [F(4,61)=3.32, p<.05]. These results suggest that nicotine can increase the ratio of dopamine/DOPAC and that this effect may be offset by pretreatment with ethanol. Tukey's *a posteriori* comparisons, however, failed to reveal any significant differences between individual treatment groups.

With respect to dopamine/DOPAC ratio (Figure 14b), two-way ANOVA revealed a significant interaction of ethanol with nicotine [$F_{(4,61)}=12.17$, $p<.001$]. Tukey's *a posteriori* comparisons indicate that subjects that received 1.0 g/kg ethanol with saline had a greater ratio of dopamine/DOPAC than did subjects that received saline only ($p<.05$). This result suggests an effect of ethanol to slow the conversion of dopamine to the dopamine metabolite, DOPAC and a possible role of nicotine to attenuate this effect.

With respect to the ratio of dopamine/HVA (Figure 14c), two-way ANOVA revealed a significant main effect of ethanol [$F_{(2,61)}=3.72$, $p<.05$], a significant main effect of nicotine [$F_{(2,61)}=6.41$, $p<.01$], and a significant nicotine x ethanol interaction [$F_{(4,61)}=6.12$, $p<.001$]. Tukey's *a posteriori* comparisons indicate that subjects that received 1.0 g/kg ethanol with saline had a higher ratio of dopamine/HVA than did subjects that received saline only ($p<.05$). This result suggests an effect of ethanol to slow the conversion of dopamine to its metabolite, HVA, and a possible role of nicotine to attenuate this effect.

To examine the relative contributions of the dopamine/l-DOPA ratio, the dopamine/DOPAC ratio, and the dopamine/HVA ratio to the variance in ASR and PPI, step-wise multiple regression correlation analysis was used. Results indicate that the dopamine/DOPAC ratio contributes significantly to the variance in the amount of PPI when the 122 dB stimulus was used [$R^2=.11$, $F(1,69)=8.45$, $p<.01$]. This result suggests that a significant negative relationship exists with respect to the ratio of dopamine/DOPAC in striatum and the amount of pre-pulse inhibition. Because the dopamine/DOPAC ratio provides an index of

dopamine turnover, this result further suggests that as the rate of conversion of dopamine to the dopamine metabolite, DOPAC increases, the amount of pre-pulse inhibition also increases.

Confirmation of Major Hypotheses

Hypothesis 3, that acute administration of 0.01 mg/kg nicotine (SC) would increase the amplitude of the acoustic startle response and would increase pre-pulse inhibition was not confirmed. There was no effect of 0.01 mg/kg nicotine on ASR or PPI when the nicotine was administered without ethanol.

Hypothesis 4, that acute administration of 0.5 mg/kg nicotine would not alter ASR or PPI relative to controls was confirmed. Acute administration of 0.5 mg/kg nicotine did not alter ASR or PPI relative to controls.

Hypothesis 5, that ethanol would decrease ASR and PPI in a linear dose-response fashion was partially confirmed. At the two doses initially proposed (1.0 g/kg and 2.0 g/kg), ethanol decreased ASR and PPI. However, the dose added during the experiment (0.5 g/kg) *increased* ASR and PPI. Therefore, the dose-effect of ethanol on ASR and PPI is more accurately described as *curvilinear* and not linear as initially hypothesized.

Hypothesis 6, that nicotine pre-treatment would attenuate ethanol's effects on PPI was partially confirmed. Although nicotine pre-treatment did not attenuate effects of 1.0 g/kg ethanol or 2.0 g/kg ethanol, nicotine pre-treatment did attenuate effects of 0.5 g/kg ethanol, providing evidence of a potentially

meaningful interaction of nicotine with ethanol to alter psychomotor performance.

Hypothesis 7, that ethanol pre-treatment would potentiate nicotine's effects to increase ASR and PPI was not confirmed. Because nicotine had no effects to increase ASR and PPI, ethanol pre-treatment could not potentiate these effects.

Hypothesis 8, that acute administration of nicotine plus ethanol together would potentiate nicotine's effects on dopamine levels in the striatum was partially confirmed. In the absence of ethanol, nicotine produced a dose-dependant dopamine depletion in striatum. Following pretreatment with ethanol, however, there was no such dopamine-depletion. This result suggests an effect of ethanol to increase dopamine availability that may be relevant to the question of why some individuals smoke more when they drink.

Discussion

The purpose of the present experiment was to examine effects of nicotine and ethanol, alone and in combination, on the amplitude of the acoustic startle response (ASR) and on the amount of pre-pulse inhibition (PPI). These measures have been interpreted to reflect processes that underlie sensory gating (Swerdlow, et al., 1986) and possibly attention (Acri, et al., 1991). When administered without nicotine, ethanol had a curvilinear dose-effect on ASR and PPI with the lowest dose of ethanol increasing ASR and PPI and the higher doses of ethanol reducing ASR and PPI (Figures 11-12). When administered with nicotine, however, these effects of ethanol were altered. Specifically, the

effects of low-dose ethanol to increase ASR and PPI were reduced in the presence of nicotine, whereas effects of high-dose ethanol to reduce ASR and PPI were unaffected by nicotine. This pattern of results suggests that nicotine can offset the effects of low-dose ethanol to increase ASR and PPI. These results also are consistent with the suggestion that nicotine and ethanol can interact to alter psychomotor function in a way that may be relevant to the tendency of some individuals to smoke more when they drink. If the increases in ASR and PPI produced by low doses of ethanol are analogous to the changes in psychomotor function that occur when people drink, and to the extent that these changes are hedonically displeasing, then it becomes easy to understand why individuals may self-administer nicotine as a means to offset these effects.

One surprising result of the present experiment is that nicotine had no effect on ASR or PPI when administered without ethanol. This result is different from the results of previous experiments that report either increases (Acri, et al., 1991; 1994) or decreases (Faraday, Rahman, Scheufele, & Grunberg, 1997) in ASR and PPI following nicotine. Future experiments, using a wider range of nicotine doses, may help to clarify the effects of nicotine on ASR and on PPI.

Biochemical analyses revealed several important interactions of nicotine with ethanol. When administered without ethanol, nicotine produced a dose-dependent depletion of dopamine and DOPAC (and to a lesser extent, I-DOPA and HVA) in striatum (fig 13). This effect of nicotine to reduce dopamine levels in striatum is consistent with reports that nicotine can deplete catecholamine

stores in central dopaminergic nerve terminals (Andersson, Fuxe, Eneroth, Harfstrand, & Agnati, 1988). When subjects were pretreated with ethanol, levels of dopamine and DOPAC were indistinguishable from controls. This result suggests an effect of ethanol to reduce nicotine-induced dopamine depletion, thereby increasing the availability of striatal dopamine. Because dopaminergic activity in striatum is an important mediator of PPI (Swerdlow, et al., 1992), this result also may suggest a mechanism by which nicotine and ethanol can interact to alter sensory-motor gating. To the extent that people smoke and drink to regulate sensory-motor function, these results may suggest an interactive effect of nicotine and ethanol that may motivate smokers to smoke and drink concurrently.

Evidence presented in the introduction of this report suggests that ethanol can increase the bioavailability of dopamine by increasing the rate of dopamine synthesis (Blomqvist et al. 1993; Masserano & Weiner 1981; Waldeck, 1974) or by decreasing the rate of dopamine metabolism (Blanchard, et al. 1993; Yoshimoto, et al. 1992). In the present experiment, these effects of ethanol were assessed by examining levels of dopamine relative to its immediate precursor, l-DOPA, to its intracellular metabolite, DOPAC, and to its extracellular metabolite, HVA. If ethanol has effects to increase the rate of dopamine synthesis, then the ratio of dopamine/l-DOPA should increase following ethanol administration. Similarly, if ethanol has effects to decrease the rate of dopamine metabolism, then the ratios of dopamine/DOPAC and of dopamine/HVA should increase following ethanol administration.

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When administered without ethanol, nicotine had a curvilinear effect on dopamine/l-DOPA ratio with the low-dose of nicotine decreasing the dopamine/l-DOPA ratio and the high-dose of nicotine increasing the dopamine/l-DOPA ratio (Figure 14a). Pretreatment with ethanol diminished these effects at each nicotine dose. More specifically, ethanol tended to attenuate the reductions in dopamine/l-DOPA ratio produced by the lowest nicotine dose and tended to attenuate the increases in dopamine/l-DOPA ratio produced by the highest nicotine dose. There was no effect of ethanol on the dopamine/l-DOPA ratio when administered without nicotine. These results appear consistent with a leftward shift in the dose-effect of nicotine by ethanol and suggest that nicotine and ethanol may interact to increase the rate dopamine synthesis in striatum. Because dopaminergic activity in striatum is known to be an important mediator of PPI, these results may suggest a mechanism by which nicotine and ethanol can interact to influence PPI. Further, these results may suggest an effect of nicotine and ethanol to alter sensory-motor function and that may motivate some individuals to smoke more when they drink.

Analysis of the relationship between dopamine and its primary metabolites, DOPAC and HVA, suggest that nicotine and ethanol may interact to alter dopamine metabolism. When administered alone, ethanol produced a dose-dependant increase in the ratio of dopamine/DOPAC and in the ratio of dopamine/HVA (Figures 14b and 14c). These results are consistent with reports that ethanol can reduce the activity of the intracellular dopamine-metabolizing enzyme MAO-B (Fowler, et al., 1996), and suggest a possible

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effect of ethanol to also reduce the activity of the extracellular dopamine-metabolizing enzyme, COMT. When administered with nicotine, effects of ethanol to increase the ratio of dopamine/DOPAC and the ratio of dopamine/HVA were reduced. This diminution in the ratio of dopamine/DOPAC and in the ratio of dopamine/HVA may reflect the nicotine-induced dopamine depletion as previously described (Andersson, et al. 1988). Future experiments should examine the activity of these different dopamine metabolizing enzymes in response to ethanol to establish their respective roles in the effects of nicotine and ethanol on dopamine turnover.

To assess the relationship between the biochemical changes induced by nicotine and ethanol and the behavioral changes induced by nicotine and ethanol, step-wise multiple regression analyses were used. Results indicate a significant negative relationship between levels of HVA in striatum and the amplitude of the acoustic startle response. To the extent that increasing levels of HVA reflect increases in dopamine turnover, and to the extent that increases in dopamine turnover imply decreases in dopaminergic activity, these results suggest that a positive relationship exists between dopaminergic activity and ASR. This interpretation is consistent with previous reports that dopamine agonists such as apomorphine, d-amphetamine, and cocaine can increase the amplitude of the acoustic startle response (Davis, et al., 1978; Davis, 1988; Harty & Davis, 1985).

Multiple regression analyses also revealed a significant negative relationship between dopamine/DOPAC ratio and PPI. To the extent that

increases in dopamine/DOPAC ratio reflect decreases in the rate of dopamine

turnover, and to the extent that decreases in dopamine turnover reflect

increases in dopaminergic activity, these results suggest that an inverse

relationship may exist between dopamine levels in striatum and the amount of

pre-pulse inhibition. This interpretation is consistent with previous reports that

dopaminergic drugs such as apomorphine (Swerdlow, et al., 1986),

amphetamine (Mansbach, Geyer, & Braff, 1988), and quinpirole (Peng,

Mansbach, Braff, & Geyer, 1989) can disrupt pre-pulse inhibition in rats.

GENERAL DISCUSSION

Experiments 1 and 2 examined effects of nicotine and alcohol on indices of reward and on indices of sensory-motor gating in rats. The aim of these experiments was to explain why many smokers smoke more when they drink. In Experiment 1, effects of nicotine to induce conditioned place preference was examined as a behavioral index of nicotine reward. In Experiment 2, subjects received nicotine and/or ethanol and the acoustic startle response (ASR), and pre-pulse inhibition of the acoustic startle response (PPI) were measured as behavioral indices of attention and sensory-motor gating. In addition, effects of nicotine and ethanol on biochemical mediators of reward and sensory-motor gating were assessed using high-performance liquid chromatography. Because nicotine is the primary, active, pharmacologic agent in tobacco, it was one of the drugs under investigation in the present research. However, it is relevant to consider that nicotine is one of several potentially active constituents of tobacco smoke (including other alkaloids and gases) and that these constituents deserve future research attention.

Results of Experiment 1 failed to support the hypothesis that repeated administration of nicotine would condition a place-preference for the nicotine-paired environment. In addition, the results of Experiment 1 failed to support the hypothesis that acute administration of ethanol would potentiate nicotine-induced place-preference. Although it is tempting to conclude that the failure of nicotine to condition a place preference reflects a general absence of reinforcement from nicotine, this interpretation is inconsistent with previous

reports using nicotine self-administration in rats (Corrigall & Coen, 1991; 1994; Corrigall, et al., 1992, 1994) and with a preponderance of experimental evidence regarding nicotine addiction in humans (see USDHHS, 1988 for review). A more likely explanation lies in the procedural differences between experiments that report place preference conditioning by nicotine (Fudala, et al., 1985; Fudala & Iwamoto, 1986; Acquas, et al., 1989; Carboni, et al., 1989) and those that do not (Clarke & Fibiger, 1987; Jorenby, Steinpreis, Sherman, & Baker, 1990). Future experiments should examine specific experimental parameters that produce conditioned place preference compared to those that do not produce conditioned place preference. In doing so, it may be possible to better design future place-preference experiments and to better understand the usefulness of this paradigm to study conditioned drug effects.

In Experiment 1, nicotine offset the locomotor depressant effects of acute ethanol treatment as indexed by the number of crosses made between the two shuttle-box chambers. Specifically, subjects that received ethanol with nicotine (on the ethanol test day) crossed between the shuttle box chambers significantly more often than did subjects that received ethanol without nicotine. These results may suggest a locomotor depressing effect of ethanol that is attenuated by subsequent administration of nicotine. To the extent that ethanol-induced locomotor depression is viewed as hedonically displeasing by the smoker, and to the extent that nicotine can offset this ethanol-induced locomotor depression, then present results may suggest one reason that some smokers smoke more when they drink. This interpretation is consistent with that

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of Shiffman, et al. (1994) who suggested that nicotine may offset some of the negative effects of ethanol and that these effects of nicotine may be responsible for the positive association between cigarette smoking and alcohol consumption

Biochemical data from Experiment 1 suggest that concurrent administration of nicotine and ethanol also was associated with a reduction in the ratio of dopamine/DOPAC in nucleus accumbens. This result was evident in all subjects, but was statistically significant only in those that had received nicotine during conditioning. This result may suggests that nicotine and alcohol can combine to increase the rate of dopamine turnover and that this effect may be dependant on prior exposure to nicotine. Multiple regression analyses, however, failed to reveal any significant relationship between effects of nicotine and ethanol on locomotor stimulation and effects of nicotine and alcohol to alter the dopamine/DOPAC ratio. Therefore, it seems unlikely that the changes in dopamine/DOPAC ratio produced by nicotine and ethanol underlie the changes in locomotor stimulation produced by nicotine and ethanol. Future experiments, examining the relationship between nicotine and ethanol-induced changes in locomotion and nicotine and ethanol-induced changes in biochemical variables, may help to clarify these mechanisms.

In conclusion, it is important to comment on several factors that may affect the interpretation of Experiment 1. First, it is important to comment on the consequences of selecting a "per-analysis" alpha level of 0.05 (or more stringent) to determine statistical significance. Setting an alpha level of 0.05 ensures a 95% likelihood that statistically significant results reflect true effects

and are not the result of Type I (experiment wise) error. However, given that 56 independent analyses of variance were performed in Experiment 1, and assuming that 5% (1 in 20) of those analyses may have occurred by chance, it is reasonable to assume that roughly 3 of the 14 statistically significant results discussed in Experiment 1 may be spurious. Therefore, it is essential that each of the statistically significant findings in Experiment 1 be replicated in future experiments to ensure the reliability of these results.

Second, it is important to comment on the usefulness of measuring the ratios of dopamine/l-DOPA, dopamine/DOPAC, and dopamine/HVA. Although these ratios have been interpreted to reflect dopamine synthesis and metabolism, it is possible that they may reflect past dopaminergic activity and are not related to the present availability of dopamine. Therefore, any interpretations regarding these ratios should be regarded tentatively. Future experiments, examining effects of nicotine and ethanol on the release of dopamine *in vivo* may help to clarify the interpretation of the ratios of dopamine/l-DOPA, dopamine/DOPAC, and dopamine/HVA.

Results of experiment 2 revealed interactions of nicotine with ethanol on ASR and on PPI. Specifically, nicotine administration attenuated effects of low-dose ethanol to increase ASR and PPI, thereby returning ASR and PPI to levels that were indistinguishable from controls. To the extent that ASR and PPI provide indices of sensory-motor gating, these data suggest that nicotine and ethanol can combine to affect normal sensory-motor function. Because it is known that a certain population of smokers smoke as a means of regulating

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sensory-motor function (USDHHS, 1988), these data may suggest one way in which nicotine and alcohol may interact to promote co-abuse.

Biochemical analyses in Experiment 2 revealed several additional interactions of nicotine with ethanol. When administered without ethanol, nicotine produced a dose dependent depletion of dopamine and of DOPAC in striatum. When administered with ethanol, however, nicotine had no such effects; levels of dopamine and DOPAC were indistinguishable from controls. Because striatum is known to be a primary neural substrate involved in PPI (Swerdlow, et al., 1992), and because ASR and PPI are known to be dopaminergically mediated (Naudin, Canu, & Costentin, 1990; Swerdlow, et al., 1992), present data may suggest a mechanism by which nicotine and ethanol can interact to alter sensory-motor function.

Analyses of the ratio of dopamine/l-DOPA (taken as an index of the rate of dopamine synthesis) in Experiment 2 indicate an effect of ethanol to shift the dose-effect of nicotine to the left. When administered without ethanol, nicotine had a curvilinear effect on dopamine/l-DOPA ratio in striatum with the low-dose of nicotine decreasing the dopamine/l-DOPA ratio and the high-dose of nicotine increasing the dopamine/l-DOPA ratio. Pretreatment with ethanol diminished these effects at each nicotine dose. More specifically, ethanol tended to attenuate the reductions in dopamine/l-DOPA ratio produced by the lowest nicotine dose and tended to attenuate the increases in dopamine/l-DOPA ratio produced by the highest nicotine dose. There was no effect of ethanol on the dopamine/l-DOPA ratio when administered without nicotine. These results

appear consistent with a leftward shift in the dose-effect of nicotine by ethanol and suggest that nicotine and ethanol may interact to increase the rate dopamine synthesis in striatum. Because dopaminergic systems are known to be important mediators of ASR and PPI (Naudin, et al., 1990; Swerdlow, et al., 1992), and because nicotine and ethanol can interact to influence dopamine synthesis in striatum, these data may suggest a mechanism by which nicotine and ethanol can interact to alter sensory-motor function, and may provide insight into why some smokers smoke more when they drink.

Analyses of the ratio of dopamine/DOPAC and of the ratio of dopamine/HVA in Experiment 2 indicate an interactive effect of nicotine and ethanol to affect dopamine turnover in striatum. When administered alone, ethanol produced a dose-dependant increase in the ratio of dopamine/DOPAC and in the ratio of dopamine/HVA. This may indicate an effect of ethanol to reduce the rate of dopamine turnover. When administered with nicotine, effects of ethanol to increase the ratio of dopamine/DOPAC and the ratio of dopamine/HVA were reduced. These results may suggest an effect of nicotine to attenuate the effects of ethanol to reduce the rate of dopamine turnover. Because dopaminergic systems are known to mediate ASR and PPI (Naudin, et al., 1990; Swerdlow, et al., 1992), this attenuation of the ethanol-induced increases in dopamine turnover in striatum suggests another way in which nicotine and ethanol may interact to influence sensory-motor function.

Multiple regression analyses in Experiment 2 revealed a significant negative relationship between levels of HVA in striatum and the amplitude of the

acoustic startle response. To the extent that increasing levels of HVA reflect increases in dopamine turnover, and to the extent that increases in dopamine turnover imply changes in dopaminergic activity, these results suggest that an positive relationship exists between dopaminergic activity and ASR. This interpretation is consistent with previous reports that dopamine agonists such as apomorphine, d-amphetamine, and cocaine can increase the amplitude of the acoustic startle response (Davis, et al., 1978; Davis, 1988; Harty & Davis, 1985).

Multiple regression analyses in Experiment 2 also revealed a significant negative relationship between dopamine/DOPAC ratio and PPI. To the extent that increases in dopamine/DOPAC ratio reflect decreases in the rate of dopamine turnover, and to the extent that decreases in dopamine turnover reflect increases in dopaminergic activity, these results suggest that an inverse relationship may exist between dopamine levels in striatum and the amount of pre-pulse inhibition. This interpretation is consistent with several reports that report effects of dopaminergic drugs such as apomorphine (Swerdlow, et al., 1986), amphetamine (Mansbach, Geyer, & Braff, 1988, and quinpirole (Peng, Mansbach, Braff, & Geyer, 1989) to disrupt pre-pulse inhibition in rats.

As in Experiment 1, it is important to comment on several factors that may influence the interpretation of present results. First, it is important to comment on the consequences of selecting a "per-analysis" alpha level of 0.05 (or more stringent) to determine statistical significance. Setting an alpha level of 0.05 ensures a probability of .95 that statistically significant results reflect true

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effects and are not the result of Type I (experiment wise) error. However, given that 39 independent analyses of variance were performed in Experiment 2, and assuming that 5% (1 in 20) of those analyses may have occurred by chance, it is reasonable to assume that roughly 2 of the 24 statistically significant results discussed in Experiment 2 may be spurious. Therefore, it is essential that each of the above findings be replicated in future experiments to ensure the reliability of the present results.

Second, it is important to comment on the usefulness of measuring the ratios of dopamine/l-DOPA, dopamine/DOPAC, and dopamine/HVA. Although these ratios have been interpreted to reflect dopamine synthesis and metabolism, it is possible that they may reflect past dopaminergic activity and are not related to the present availability of dopamine. Therefore, any interpretations regarding these ratios should be regarded tentatively. Future experiments, examining effects of nicotine and ethanol on the release of dopamine *in vivo* may help to clarify the interpretation of the ratios of dopamine/l-DOPA, dopamine/DOPAC, and dopamine/HVA.

Finally, it is important to note that analyses of biochemical results in Experiment 2 were performed on tissue that was multiply sampled from several representative subjects from each drug treatment group. Therefore, the within-groups error variance that contributed to the present ANOVA model is a combination of the variance in the population of and the variance in the HPLC assay procedure. Because it is possible that such sampling may have minimized the proportion of variance attributed to within-groups factors (thereby

increasing the value, F, in subsequent ANOVA), these biochemical results may be viewed as preliminary.

In summary, the present dissertation research used animal models to assess behavioral and biochemical factors that may contribute to the tendency of some smokers to smoke more when they drink. Results of Experiment 1 suggest that nicotine can offset some of the locomotor depressant effects of ethanol and that nicotine and ethanol can combine to reduce the ratio of dopamine/DOPAC in nucleus accumbens. Results of experiment 2 suggest that nicotine and ethanol can interact to influence ASR and PPI, and that nicotine and ethanol can combine to alter levels of dopamine and DOPAC in striatum, and to alter the ratios of dopamine/l-DOPA, dopamine/DOPAC, and dopamine/HVA in striatum. Because the behavioral variables used presently provide indices of sensory motor function, and because some smokers may smoke to regulate sensory-motor performance, these data suggest a behavioral mechanism that may motivate smokers to smoke more when they drink. In addition, because dopaminergic activity in nucleus accumbens and striatum is known mediate nicotine reward and sensory-motor function, respectively, these data also suggest possible biochemical mechanisms that may motivate smokers to smoke more when they drink.

TABLES

Table 1.   Experimental Timeline - Experiment 1

Unconditioned preference test (days 1-3)

• All subjects were given free access to both chambers of the shuttle-box to establish the "unbiased" place preference.

Place preference conditioning (days 4-8)

• Place preference for nicotine was induced by pairing acute injections of nicotine with one chamber and acute injections of saline with the other.

Assessment of place preference (day 9)

• Subjects were given free access to both chambers of the shuttle box but did not receive drug. The time spent in each was recorded as an index of place conditioning.

Ethanol test (day 10)

• Subjects received ethanol or saline and were given access to both chambers of the shuttle-box. The time spent in each was recorded as an index of place preference conditioning. Immediately after testing, subjects were sacrificed by decapitation. Brains were removed and frozen for later assay.

Table 2.   Experimental Design - Experiment 1

Days 1-3	Days 4-8	Day 9	Day 10
Initial Place Preference Test	Place Preference Established with Nicotine	Place Preference Testing	Ethanol Test and Sacrifice
Group 1- No Drug Treatment	0.8 mg/kg nicotine (SC)	no drug treatment	1.0 g/kg ethanol (IP)
Group 2- No Drug Treatment	0.8 mg/kg nicotine (SC)	no drug treatment	6.6 ml/kg saline (IP)
Group 3- No Drug Treatment	0.8 mg/kg nicotine (SC)	no drug treatment	1.0 g/kg ethanol + 0.8 mg/kg nicotine
Group 4- No Drug Treatment	0.8 ml/kg saline (SC)	no drug treatment	6.6 ml/kg saline (IP)
Group 5- No Drug Treatment	0.8 ml/kg saline (SC)	no drug treatment	1.0 g/kg ethanol (IP)
Group 6- No Drug Treatment	0.8 ml/kg saline (SC)	no drug treatment	1.0 g/kg ethanol + 0.8 mg/kg nicotine

n = 16 subjects per group

Table 3. Case-Control Pairings

Each subject in the "Case" column received nicotine during conditioning. Subjects in the corresponding "control" column received saline only during conditioning.			
CASE	CORRESPONDING CONTROL	CASE	CORRESPONDING CONTROL
1	9	49	45
2	10	50	46
3	7	51	32
4	19	52	8
5	11	53	59
6	12	54	60
13	22	61	69
14	21	62	70
15	31	63	67
16	20	64	68
17	24	65	71
18	23	66	72
25	33	73	82
26	34	74	94
27	44	75	79
28	56	76	80
29	35	77	83
30	36	78	84
37	57	85	93
38	58	86	81
39	43	87	91
40	55	88	92
41	47	89	95
42	48	90	96

Table 4. Experiment 1. Results of Repeated-Measures Analyses (days 3-10). Means ± S.E.M. presented for analyses with significant F values.

Effects of time on conditioned place preference

Nicotine-treated subjects
that were conditioned in the $[F(2,40)=0.27, \text{n.s.}]$
preferred chamber

Nicotine-treated subjects
that were conditioned in the $[F(2,42)=1.22, \text{n.s.}]$
non-preferred chamber

Saline-treated subjects
that were conditioned in the $[F(2,40)=1.63, \text{n.s.}]$
preferred chamber

Saline-treated subjects
that were conditioned in the $[F(2,46)=2.25, \text{n.s.}]$
non-preferred chamber

Effects of time on latency to cross
(subjects began test sessions in the saline-paired side)

Nicotine-treated subjects
that were conditioned in the $[F(2,28)=2.92, \text{n.s.}]$
preferred chamber

Nicotine-treated subjects
that were conditioned in the $[F(2,10)=6.10, p<.05]$
non-preferred chamber

Latency Day 3 = 12.3 ± 6.4
Latency Day 9 = 19.2 ± 5.5
Latency Day 10= 3.3 ± 0.8

Saline-treated subjects
that were conditioned in the $[F(2,26)=1.26, \text{n.s.}]$
preferred chamber

Table 4. (cont.)

Saline-treated subjects
that were conditioned in the $[F(2,16)=0.11, n.s.]$
non-preferred chamber

Effects of time on latency to cross
(subjects began test sessions in the nicotine-paired side)

Nicotine-treated subjects
that were conditioned in the $[F(2,12)=0.87, n.s.]$
preferred chamber

Nicotine-treated subjects
that were conditioned in the $[F(2,28)=0.24, n.s.]$
non-preferred chamber

Saline-treated subjects
that were conditioned in the $[F(2,12)=1.1, n.s.]$
preferred chamber

Saline-treated subjects
that were conditioned in the $[F(2,26)=1.11, n.s.]$
non-preferred chamber

Effects of time on the number of crossings between chambers

Nicotine-treated subjects
that were conditioned in the $[F(2,44)=9.80, p<.05]$
preferred chamber

Crossings Day 3 = 80.0 ± 8.1
Crossings Day 9 = 98.4 ± 6.33
Crossings Day 10= 62.8 ± 6.4

Table 4. (cont.)

Nicotine-treated subjects
that were conditioned in the $[F(2,36)=3.90, p<.05]$
non-preferred chamber

Crossings Day 3 = 91.9 ± 5.7
Crossings Day 9 = 93.4 ± 6.5
Crossings Day 10= 64.9 ± 8.5

Saline-treated subjects
that were conditioned in the $[F(2,40)=14.6, p<.05]$
preferred chamber

Crossings Day 3 = 76.5 ± 5.5
Crossings Day 9 = 73.7 ± 5.4
Crossings Day 10= 44.3 ± 5.2

Saline-treated subjects
that were conditioned in the $[F(2,44)=20.7, p<.05]$
non-preferred chamber

Crossings Day 3 = 81.3 ± 7.6
Crossings Day 9 = 106.2 ± 6.1
Crossings Day 10= 47.0 ± 6.9

Table 5. Results of Between Subjects ANOVA (Experiment 1)

Conditioned Place Preference on place preference test day

Effects of nicotine-treatment among subjects that were conditioned in the preferred chamber (nic. vs saline)	[F(1,44)=0.46, n.s.]
Effects of nicotine-treatment among subjects that were conditioned in the non-preferred chamber (nic. vs saline)	[F(1,48)=1.00, n.s.]

Latency to cross on place preference test day (subjects began test sessions in the saline-paired side)

Effects of nicotine-treatment among subjects that were conditioned in the preferred chamber (nic. vs saline)	[F(1,32)=1.60, n.s.]
Effects of nicotine-treatment among subjects that were conditioned in the non-preferred chamber (nic. vs saline)	[F(1,16)=5.19, p<.05] Saline: 7.7 ± 1.9 Nicotine: 19.3 ± 5.5

Latency to cross on place preference test day (subjects began test sessions in the nicotine-paired side)

Effects of nicotine-treatment among subjects that were conditioned in the preferred chamber (nic. vs saline)	[F(1,13)=0.00, n.s.]

Table 5. (cont.)

Effects of nicotine-treatment
among subjects that were
conditioned in the non-preferred   [$F_{(1,32)}$=0.63, n.s.]
chamber (nic. vs saline)

Number of crossings on place preference test day

Effects of nicotine-treatment
among subjects that were   [$F_{(1,45)}$=8.85, p<.05]
conditioned in the preferred
chamber (nic. vs saline)   Saline: 73.7 ± 5.3
                                           Nicotine: 98.4 ± 6.3

Effects of nicotine-treatment
among subjects that were   [$F_{(1,46)}$=2.10, n.s.]
conditioned in the non-preferred
chamber (nic. vs saline)

Conditioned Place Preference on Ethanol Test day

Effects of drug treatment
among subjects that were   [$F_{(2,23)}$=0.89, n.s.]
conditioned with nicotine
in the preferred chamber

Effects of drug treatment
among subjects that were   [$F_{(2,22)}$=0.65, n.s.]
conditioned with nicotine
in the non-preferred chamber

Table 5. (cont.)

Effects of drug treatment
among subjects that were
conditioned with saline
in the preferred chamber

$[F_{(2,22)}=0.04$, n.s.]

Effects of drug treatment
among subjects that were
conditioned with saline
in the non-preferred chamber

$[F_{(2,24)}=1.68$, n.s.]

Latency to cross on the Ethanol test day
(subjects began test sessions in the saline-paired side)

Effects of drug treatment
among subjects that were
conditioned with nicotine
in the preferred chamber

$[F_{(2,15)}=0.16$, n.s.]

Effects of drug treatment
among subjects that were
conditioned with nicotine
in the non-preferred chamber

$[F_{(2,5)}=10.54$, $p<.05]$

Saline:	1.6 ± 0.5
ETOH:	5.1 ± 0.8
ETOH+Nic:	1.6 ± 0.5

Effects of drug treatment
among subjects that were
conditioned with saline
in the preferred chamber

$[F_{(2,13)}=2.85$, n.s.]

Effects of drug treatment
among subjects that were
conditioned with saline
in the non-preferred chamber

$[F_{(2,8)}=2.32$, n.s.]

Table 5. (cont.)

Latency to cross on the Ethanol test day
(subjects began test sessions in the nicotine-paired side)

Effects of drug treatment
among subjects that were
conditioned with nicotine
in the preferred chamber

$[F_{(2,6)}=0.11, n.s.]$

Effects of drug treatment
among subjects that were
conditioned with nicotine
in the non-preferred chamber

$[F_{(2,14)}=2.69, n.s.]$

Effects of drug treatment
among subjects that were
conditioned with saline
in the preferred chamber

$[F_{(2,6)}=1.66, n.s.]$

Effects of drug treatment
among subjects that were
conditioned with saline
in the non-preferred chamber

$[F_{(2,13)}=4.5, p<.05]$

Saline:	1.5 ± 0.5
ETOH:	0.8 ± 0.2
ETOH+Nic:	6.1 ± 2.6

Number of Crossings on Ethanol Test day

Effects of drug treatment
among subjects that were
conditioned with nicotine
in the preferred chamber

$[F_{(2,23)}=4.70, p<.05]$

Saline:	70.6 ± 8.0
ETOH:	39.3 ± 9.2
ETOH+Nic:	78.6 ± 11.3

Effects of drug treatment
among subjects that were
conditioned with nicotine
in the non-preferred chamber

$[F_{(2,21)}=11.2, p<.05]$

Saline:	100.4 ± 15.7
ETOH:	29.0 ± 7.6
ETOH+Nic:	65.1 ± 6.5

Effects of drug treatment
among subjects that were
conditioned with saline
in the preferred chamber

$[F_{(2,23)}=13.28, p<.05]$

Saline:	71.5 ± 8.4
ETOH:	16.1 ± 5.9
ETOH+Nic:	41.8 ± 7.6

Table 5. (cont.)

Effects of drug treatment
among subjects that were
conditioned with saline
in the non-preferred chamber

$[F_{(2,25)}=10.56, p<.05]$

Saline:	76.8 ± 9.5
ETOH:	19.7 ± 4.8
ETOH+Nic:	48.1 ± 11.7

Effects of Drug Treatment (administered on the ethanol test day) on Central Neurochemistry

Effects of treatment in
subjects that received
saline during place-
preference conditioning

Dopamine:
  $[F_{(5,94)}=0.75,$ n.s.]
l-DOPA:
  $[F_{(5,94)}=1.99,$ n.s.]
DOPAC:
  $[F_{(5,94)}=1.49,$ n.s.]
HVA:
  $[F_{(5,94)}=1.32,$ n.s.]
Dopamine/DOPAC:
  $[F_{(5,94)}=2.06,$ n.s.]
Dopamine/HVA:
  $[F_{(5,94)}=1.24,$ n.s.]
Dopamine/l-DOPA:
  $[F_{(5,93)}=0.24,$ n.s.]

Effects of treatment in
subjects that received
Nicotine during place-
preference conditioning

Dopamine:
  $[F_{(5,94)}=0.31,$ n.s.]
l-DOPA:
  $[F_{(5,94)}=0.79,$ n.s.]
DOPAC:
  $[F_{(5,94)}=0.87,$ n.s.]
HVA:
  $[F_{(5,94)}=0.12,$ n.s.]
Dopamine/DOPAC:
  $[F_{(5,94)}=3.48, p<.05]$
Dopamine/HVA:
  $[F_{(5,94)}=1.39,$ n.s.]
Dopamine/l-DOPA:
  $[F_{(5,93)}=0.60,$ n.s.]

Table 6.   Correlation Matrix for Experiment 1

Subjects Received Saline During Conditioning

	DA	I-DOPA	DOPAC	HVA	DA/I-DOPA	DA/DOPAC	DA/HVA
Preference	R=-.06	R=.17	R=.05	R=.23	R=-.13	R=-.11	R=-.20
Latency	R=-.01	R=.06	R=.01	R=.03	R=-.06	R=.03	R=-.03
Crossings	R=-.06	R=.16	R=.01	R=.09	R=-.16	R=-.07	R=-.14

Subjects Received Nicotine During Conditioning

	DA	I-DOPA	DOPAC	HVA	DA/I-DOPA	DA/DOPAC	DA/HVA
Preference	R=.01	R=-.08	R=.02	R=-.07	R=.07	R=.05	R=.12
Latency	R=-.04	R=-.05	R=-.02	R=.02	R=-.01	R=-.08	R=-.11
Crossings	R=.16	R=.14	R=.10	R=.09	R=-.06	R=.10	R=.05

Table 7.   Experimental Design - Experiment 2

2nd Injection

1st Injection	saline	0.01 mg/kg nicotine (SC)	0.5 mg/kg nicotine (SC)
saline	n=8	n=8	n=8
0.5 g/kg ethanol (IP)	n=8	n=8	n=8
1.0 g/kg ethanol (IP)	n=8	n=8	n=8
2.0 g/kg ethanol (IP)	n=8	n=8	n=8

N=96

Table 8. Listing of Two-Way ANOVA Results (Experiment 2). Means ± S.E.M. provided for analyses with significant F vaules.

ASR using the 112 dB stimulus

Ethanol $[F_{(3,79)}=9.62, p<.05]$
Nicotine $[F_{(2,79)}=2.41, n.s.]$
Ethanol x Nicotine $[F_{(6,79)}=2.85, p<.05]$

2nd Injection

1st Injection	saline	0.01 mg/kg nicotine	0.5 mg/kg nicotine
saline	58.5 ± 12.2	47.1 ± 9.0	50.9 ± 9.9
0.5 g/kg ethanol	109.3 ± 30.4	48.1 ± 11.0	38.0 ± 9.8
1.0 g/kg ethanol	25.0 ± 6.5	37.8 ± 7.2	35.1 ± 6.4
2.0 g/kg ethanol	14.7 ± 2.5	20.1 ± 3.8	11.6 ± 2.0

ASR using the 122 dB stimulus

Ethanol $[F_{(3,81)}=9.38, p<.05]$
Nicotine $[F_{(2,81)}=3.71, p<.05]$
Ethanol x Nicotine $[F_{(6,81)}=3.51, p<.05]$

2nd Injection

1st Injection	saline	0.01 mg/kg nicotine	0.5 mg/kg nicotine
saline	165.3 ± 19.4	108.4 ± 8.0	151.0 ± 26.0
0.5 g/kg ethanol	205.5 ± 29.7	85.4 ± 12.8	112.4 ± 27.19.7
1.0 g/kg ethanol	90.9 ± 16.3	87.3 ± 9.5	100.3 ± 10.3
2.0 g/kg ethanol	56.4 ± 14.9	91.1 ± 23.5	56.5 ± 12.0

Table 8. (cont.)

PPI using the 112 dB stimulus

Ethanol $[F_{(3,79)}=4.37, p<.05]$
Nicotine $[F_{(2,79)}=1.30, n.s.]$
Ethanol x Nicotine $[F_{(6,79)}=1.37, n.s.]$

<div align="center">2nd Injection</div>

1st Injection	saline	0.01 mg/kg nicotine	0.5 mg/kg nicotine
saline	21.1 ± 5.8	19.7 ± 4.0	23.3 ± 5.2
0.5 g/kg ethanol	36.1 ± 17.4	15.3 ± 5.3	12.0 ± 3.8
1.0 g/kg ethanol	9.7 ± 4.2	16.0 ± 3.7	5.3 ± 2.2
2.0 g/kg ethanol	5.0 ± 1.9	10.1 ± 1.8	2.4 ± 1.7

PPI using the 122 dB stimulus

Ethanol $[F_{(3,81)}=3.32, p<.05]$
Nicotine $[F_{(2,81)}=0.81, n.s.]$
Ethanol x Nicotine $[F_{(6,81)}=1.72, n.s.]$

<div align="center">2nd Injection</div>

1st Injection	saline	0.01 mg/kg nicotine	0.5 mg/kg nicotine
saline	75.8 ± 15.8	27.1 ± 6.1	76.1 ± 18.5
0.5 g/kg ethanol	62.9 ± 31.1	32.3 ± 11.9	45.4 ± 14.6
1.0 g/kg ethanol	22.1 ± 11.5	46.8 ± 6.1	38.9 ± 6.3
2.0 g/kg ethanol	16.3 ± 3.4	31.0 ± 11.1	25.1 ± 5.2

Table 8. (cont.)

Percent PPI using the 112 dB stimulus

Ethanol	[$F_{(3,79)}$=1.67, n.s.]
Nicotine	[$F_{(2,79)}$=2.50, n.s.]
Ethanol x Nicotine	[$F_{(6,79)}$=1.26, n.s.]

Percent PPI using the 122 dB stimulus

Ethanol	[$F_{(3,83)}$=0.43, n.s.]
Nicotine	[$F_{(2,83)}$=0.90, n.s.]
Ethanol x Nicotine	[$F_{(6,83)}$=1.63, n.s.]

Effects of Drug Treatment on Central Neurochemistry

Ethanol

Dopamine	[$F_{(2,61)}$=16.15, p<.001]
l-DOPA:	[$F_{(2,61)}$=3.59, p<.05]
DOPAC:	[$F_{(2,61)}$=8.06, p<.01]
HVA:	[$F_{(2,61)}$=3.56, p<.05]
Dopamine/DOPAC:	[$F_{(2,61)}$=2.51, n.s.]
Dopamine/HVA:	[$F_{(2,61)}$=3.72, p<.05]
Dopamine/l-DOPA:	[$F_{(2,61)}$=0.90, n.s.]

Nicotine

Dopamine:	[$F_{(2,61)}$=2.40, n.s.]
l-DOPA:	[$F_{(2,61)}$=6.14, p<.01]
DOPAC:	[$F_{(2,61)}$=0.40, n.s.]
HVA:	[$F_{(2,61)}$=4.30, p<.05]
Dopamine/DOPAC:	[$F_{(2,61)}$=2.34, n.s.]
Dopamine/HVA:	[$F_{(2,61)}$=6.41, p<.01]
Dopamine/l-DOPA:	[$F_{(2,61)}$=4.16, p<.05]

Ethanol x Nicotine

Dopamine	[$F_{(4,61)}$=3.69, p<.01]
l-DOPA:	[$F_{(4,61)}$=4.80, p<.01]
DOPAC:	[$F_{(4,61)}$=12.32, p<.001]
HVA:	[$F_{(4,61)}$=8.07, p<.001]
Dopamine/DOPAC:	[$F_{(4,61)}$=12.17, p<.001]
Dopamine/HVA:	[$F_{(4,61)}$=6.12, p<.001]
Dopamine/l-DOPA:	[$F_{(4,61)}$=3.324, p<.05]

Table 8. (cont.)

Means Table for Biochemical Data From Experiment 2 (Means ± S.E.M.)

Tx Group	Da	I-DOPA	DOPAC	HVA	Da / DOPAC	Da / I-DOPA	Da / HVA
saline + saline	1163.0 ± 18.6	2.0 ± 0.4	254.6 ± 10.5	122.1 ± 10.4	4.6 ± 0.02	598.4 ± 134.1	9.6 ± 0.1
saline + 0.01 mg/kg nicotine	1046.8 ± 35.4	2.1 ± 0.4	217.1 ± 16.5	132.7 ± 5.3	4.8 ± 0.2	504.6 ± 24.5	9.1 ± 1.1
saline + 0.5 mg/kg nicotine	918.9 ± 127.7	1.3 ± 0.06	172.4 ± 2.6	92.4 ± 5.3	5.3 ± 0.2	703.7 ± 19.1	10.1 ± 0.6
0.5 g/kg ethanol + saline	937.0 ± 78.1	1.7 ± 0.2	186.8 ± 16.8	98.9 ± 8.0	5.1 ± 0.2	551.8 ± 33.6	9.5 ± 0.4
0.5 g/kg ethanol + 0.01 mg/kg nicotine	982.5 ± 43.6	1.8 ± 0.1	185.3 ± 8.2	105.1 ± 6.9	5.3 ± 0.2	575.4 ± 46.6	5.3 ± 0.2
0.5 g/kg ethanol + 0.5 mg/kg nicotine	998.0 ± 30.1	1.8 ± 0.1	211.2 ± 12.9	110.9 ± 4.8	4.8 ± 0.2	578.2 ± 25.7	9.1 ± 0.4
1.0 g/kg ethanol + saline	1162.4 ± 27.0	1.8 ± 0.01	194.3 ± 4.6	98.3 ± 2.2	6.0 ± 0.003	642.9 ± 10.0	11.8 ± 0.005
1.0 g/kg ethanol + 0.01 mg/kg nicotine	1216.2 ± 37.6	2.2 ± 0.2	249.6 ± 17.7	132.7 ± 5.3	4.9 ± 0.2	557.8 ± 25.9	9.2 ± 0.08
1.0 g/kg ethanol + 0.5 mg/kg nicotine	1171.2 ± 36.3	1.7 ± 0.3	236.8 ± 10.2	114.4 ± 4.5	5.0 ± 0.3	587.0 ± 50.8	10.4 ± 0.6

Table 9. Correlation Matrix for Experiment 2

112 dB Stimulus

	DA	I-DOPA	DOPAC	HVA	DA/I-DOPA	DA/DOPAC	DA/HVA
ASR	R=-.29	R=-.21	R=-.21	R=-.33	R=.05	R=.06	R=.22
PPI	R=-.32	R=-.15	R=-.24	R=-.33	R=-.04	R=-.05	R=.02
%PPI	R=.02	R=.02	R=.02	R=-.09	R=-.05	R=-.05	R=.08

122 dB Stimulus

	DA	I-DOPA	DOPAC	HVA	DA/I-DOPA	DA/DOPAC	DA/HVA
ASR	R=-.31	R=-.21	R=-.21	R=-.33	R=.06	R=-.06	R=.14
PPI	R=-.16	R=-.07	R=.06	R=-.33	R=-.04	R=-.06	R=-.24
%PPI	R=.05	R=.01	R=.21	R=.17	R=.01	R=-.30	R=-.20

FIGURES
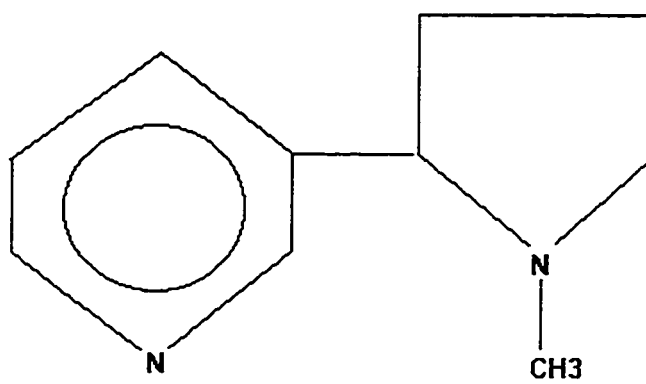
Figure 1.
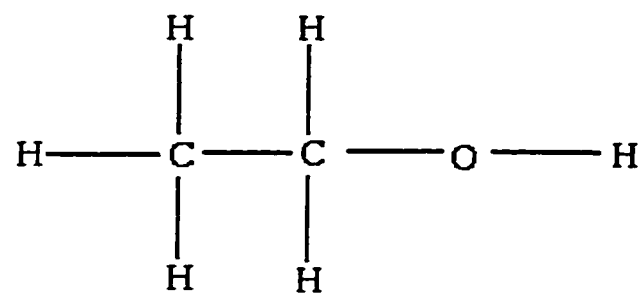
Figure 2.
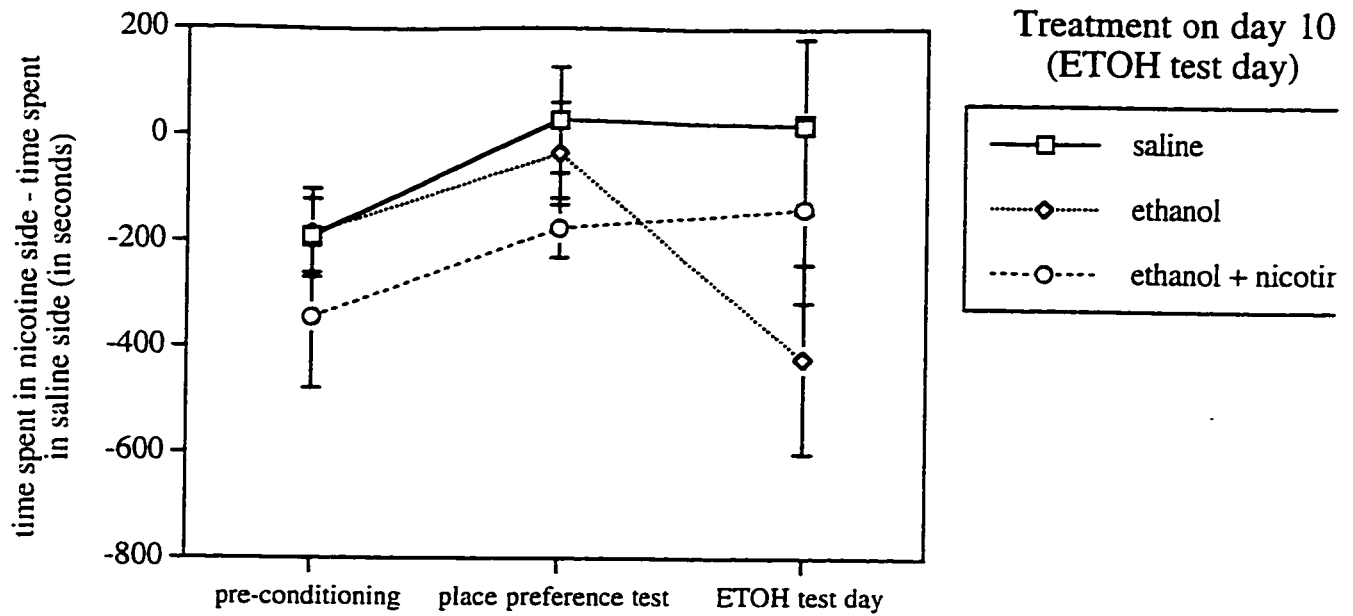
Figure 3a. Place preference among subjects that received saline during conditioning (non-preferred side designated as nicotine side; Means and SEM)
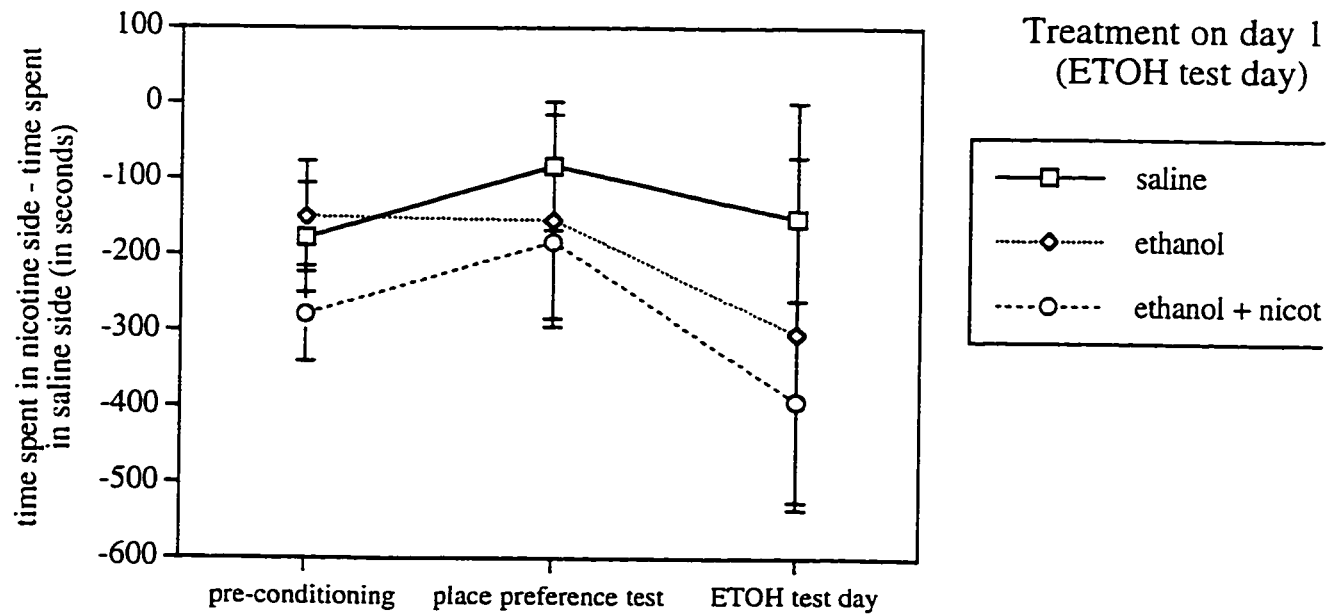
Figure 3b. Place preference among subjects that received nicotine during conditioning (non-preferred side designated as nicotine side; Means and SEM)

Treatment on day 10 (ETOH test day)

- □ saline
- ◇ ethanol
- ○ ethanol + nicoi
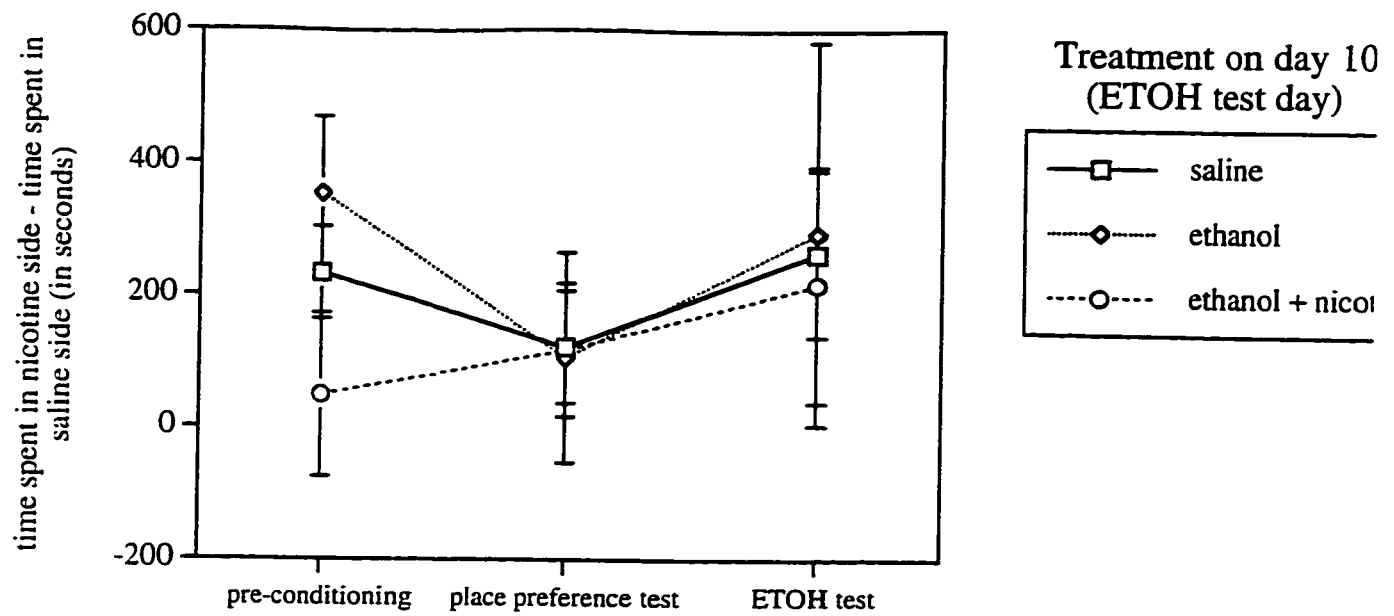
Figure 4a. Place preference among subjects that received saline during conditioning (preferred side designated as nicotine side; Means and SEM)

Treatment on day 1 (ETOH test day)

- □ saline
- ◇ ethanol
- ○ ethanol + nicc
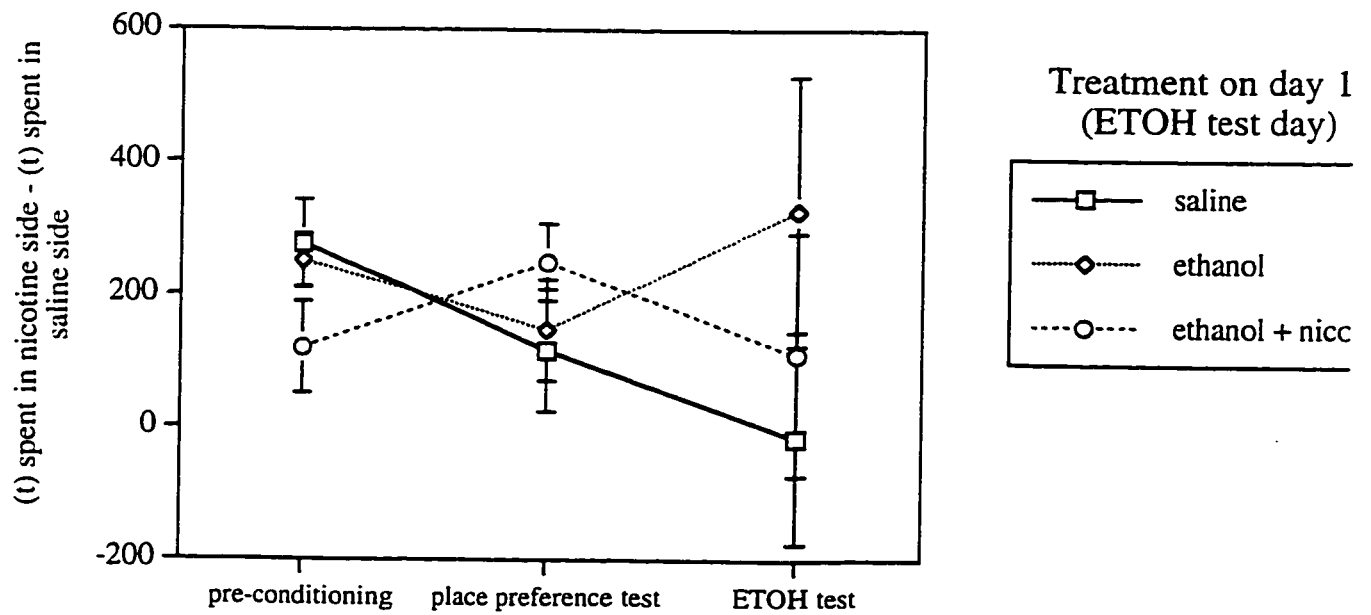
Figure 4b. Place preference among subjects received nicotine during conditioning (preferred side designated as nicotine side; Means and SEM)
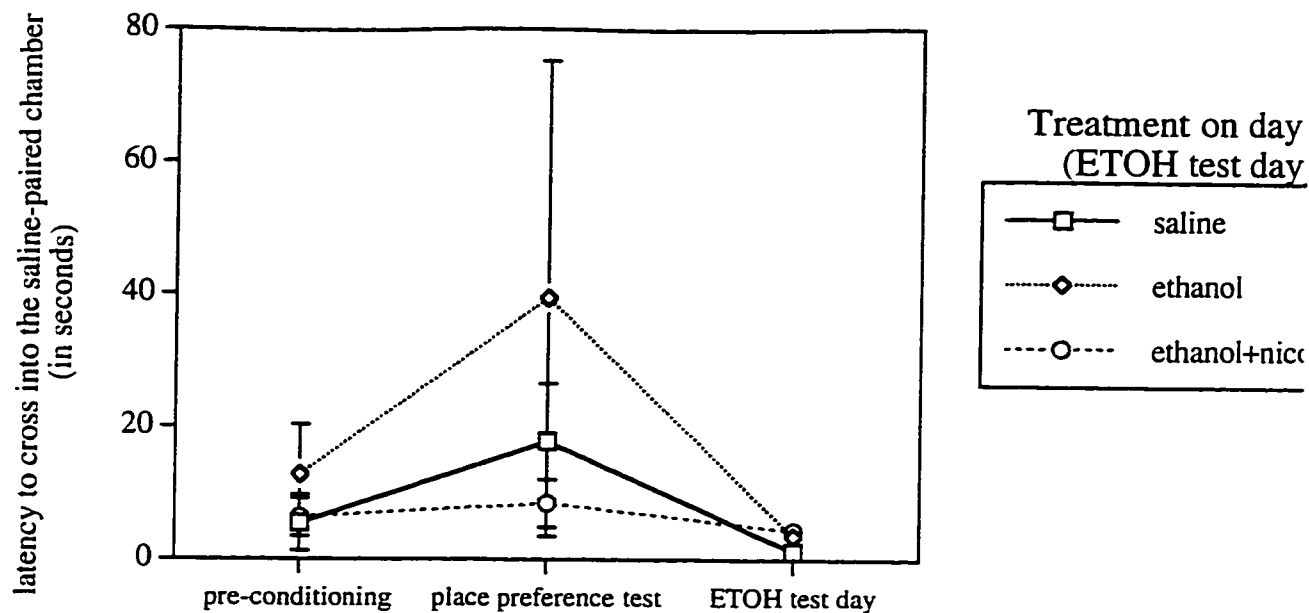
Figure 5a. latency to cross into the saline-paired chamber
(subjects received saline during conditioning; Means and SEM)
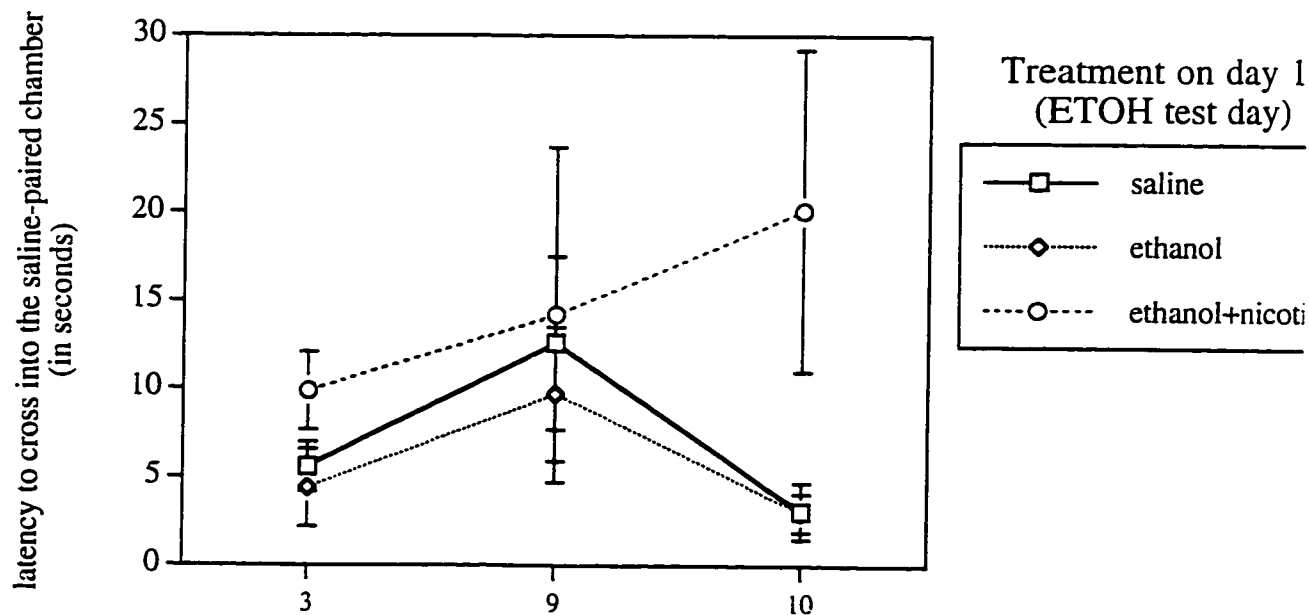
Figure 5b. latency to cross into the saline-paired chamber
(subjects received nicotine during conditioning; Means and SEM)
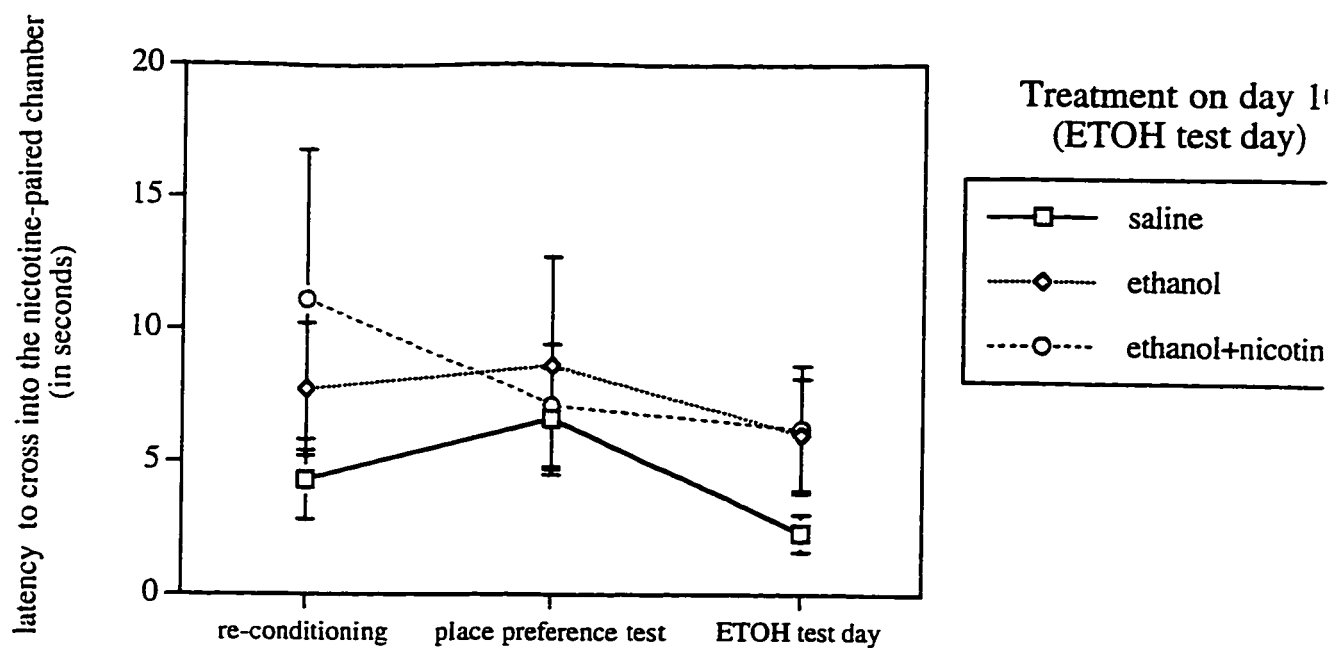
Figure 6a. latency to cross into the nicotine-paired chamber
(subjects received saline during conditioning; Means and SEM)
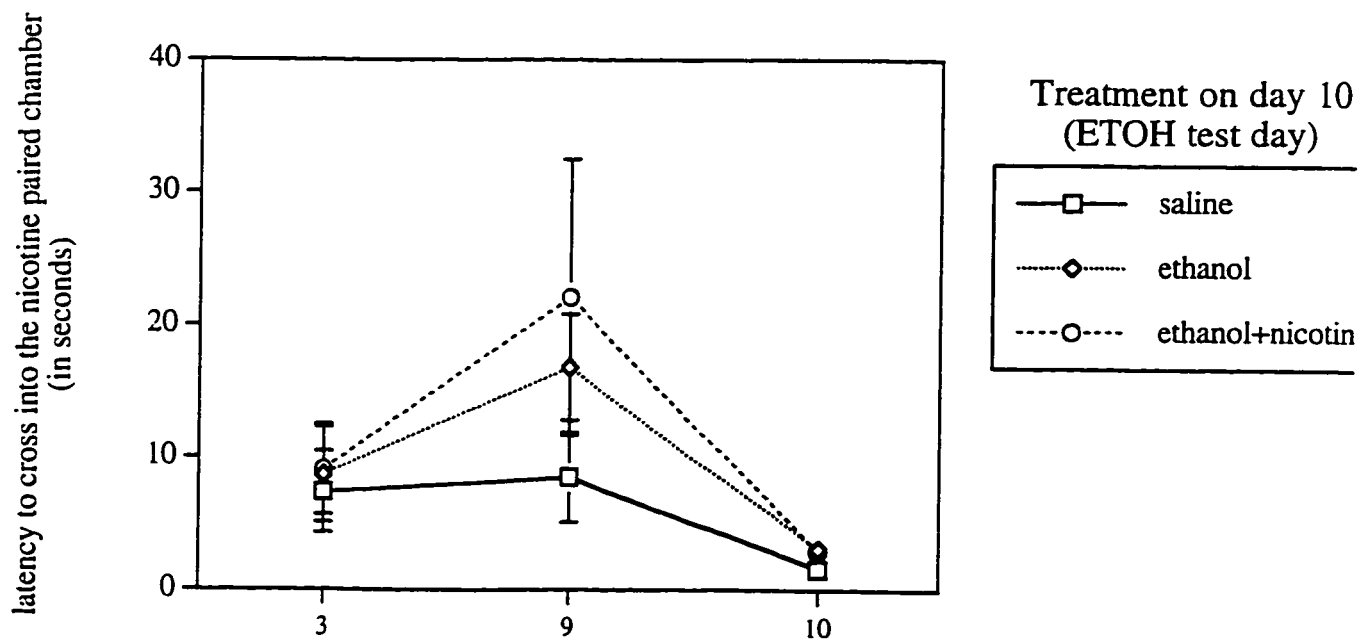
Figure 6b. latency to cross into the nicotine-paired chamber
(subjects received nicotine during conditioning; Means and SEM)
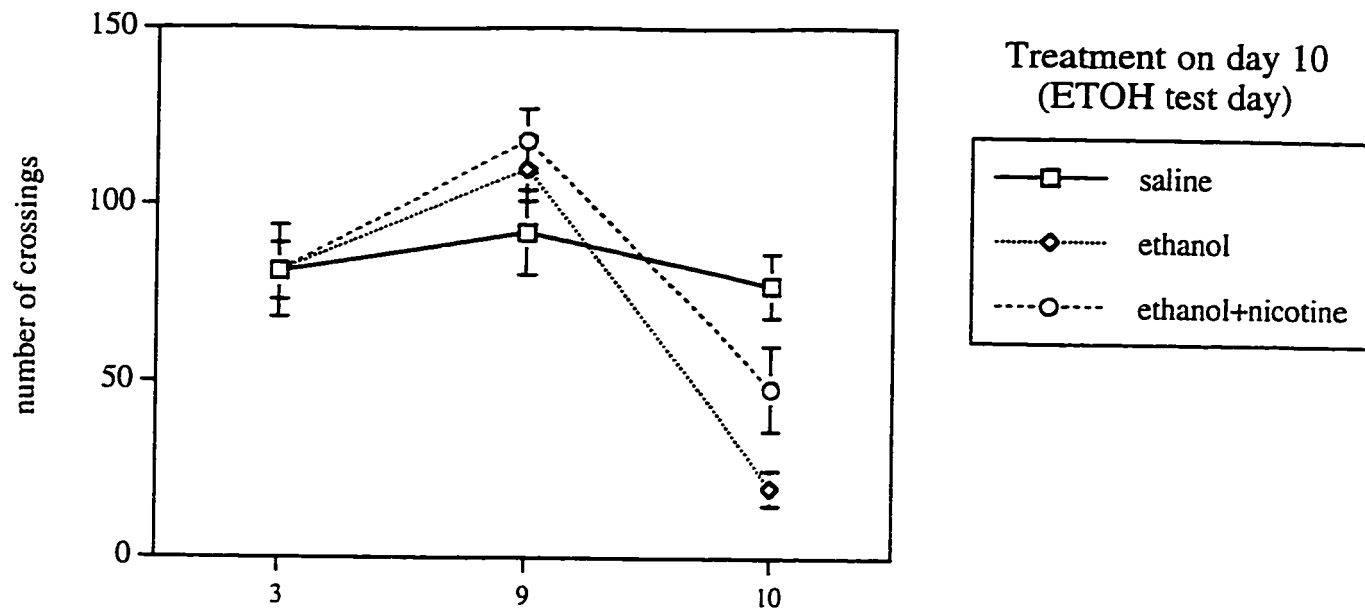
Figure 7a. crosses between chambers - subjects received saline in
the non-preferred chamber during conditioning (Means and SEM)
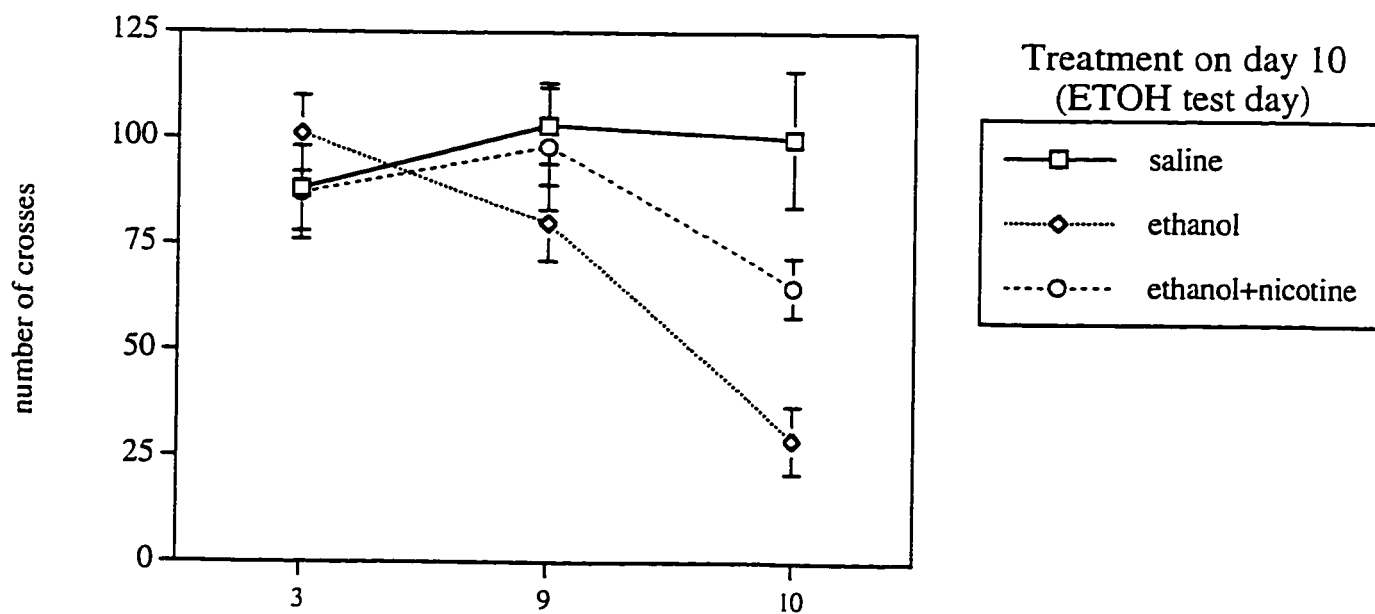
Figure 7b. crosses between chambers - subjects received nicotine in
the non-preferred chamber during conditioning (Means and SEM)
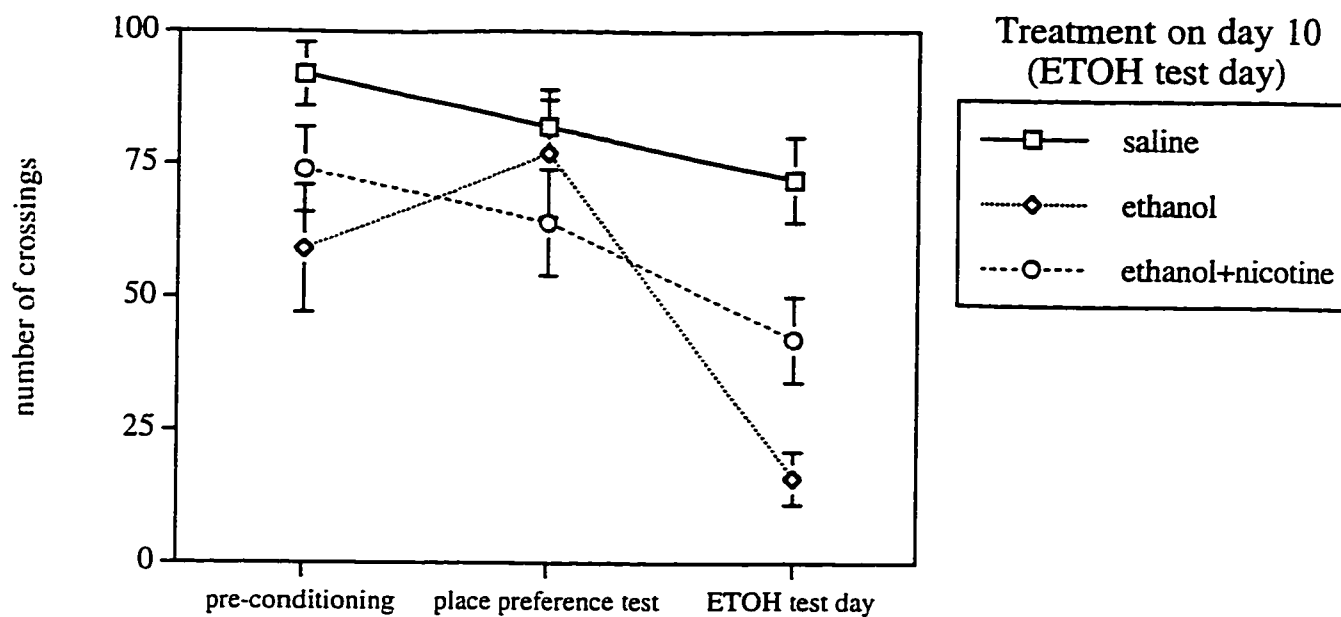
Figure 8a. number of crossings - subjects received saline in
the preferred chamber during conditioning (Means and SEM)
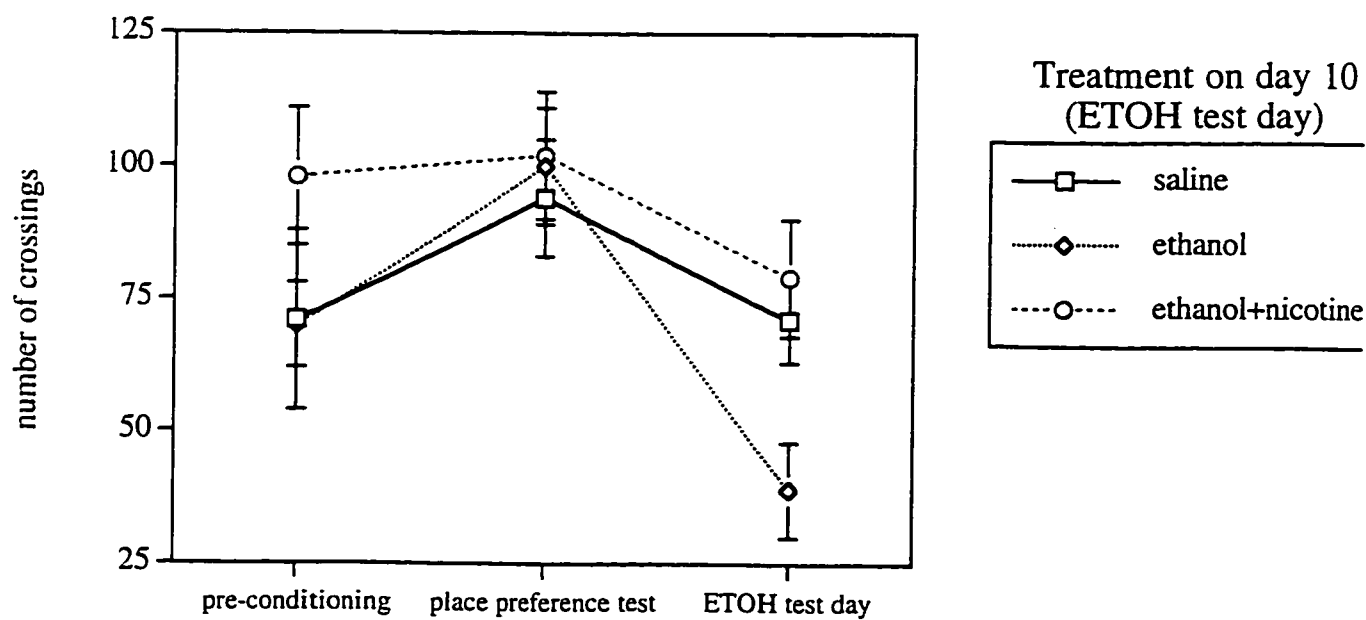
Figure 8b. number of crossings - subjects received nicotine in
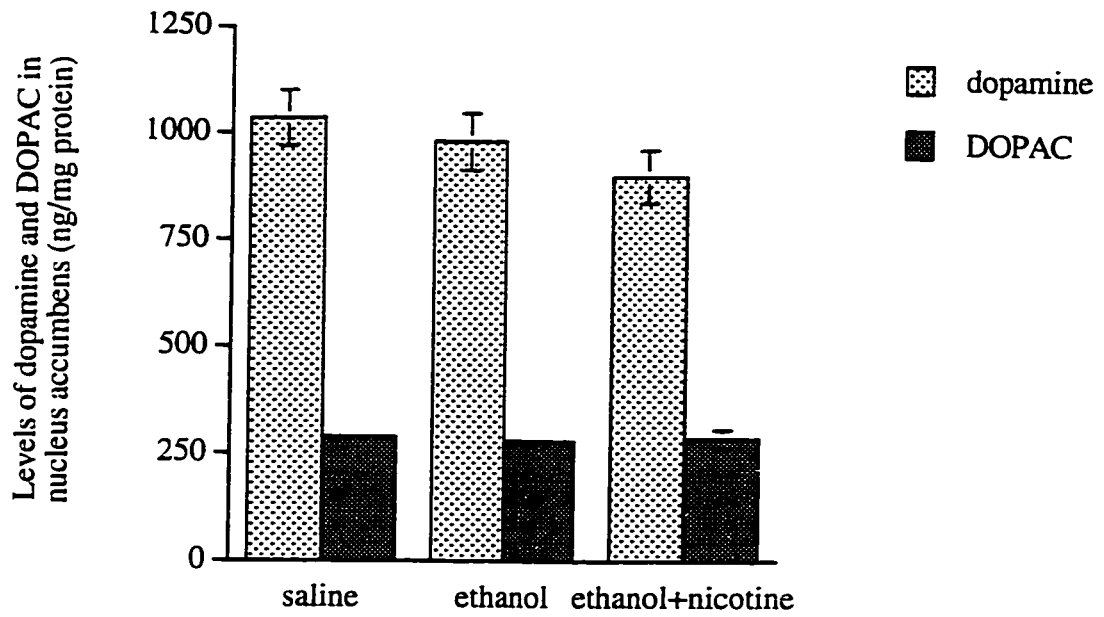the preferred chamber during conditioning (Means and SEM)
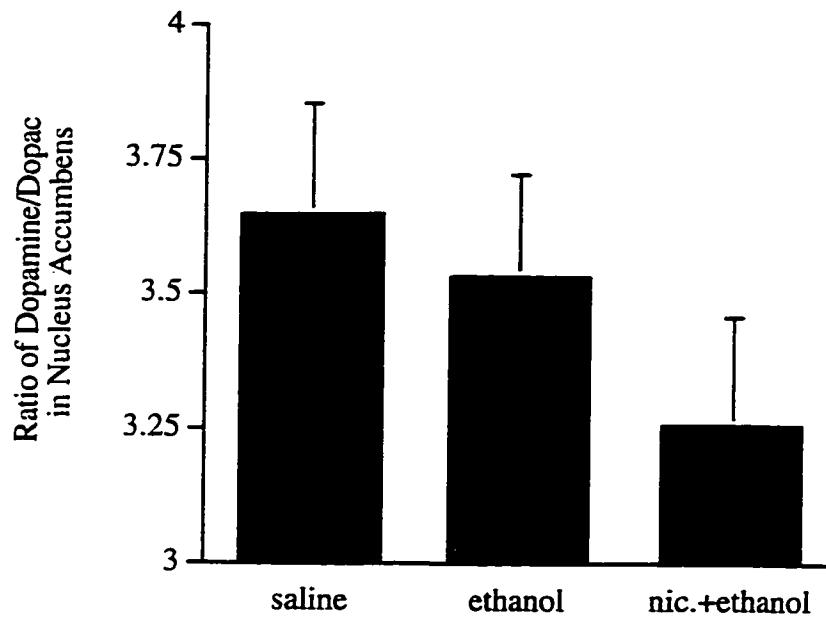
Figure 9a. Ratio of Dopamine/DOPAC in nucleus accumbens following treatment with saline, ethanol. or nicotine+ethanol. Subjects received saline during conditioning. (Means and S.E.M.)
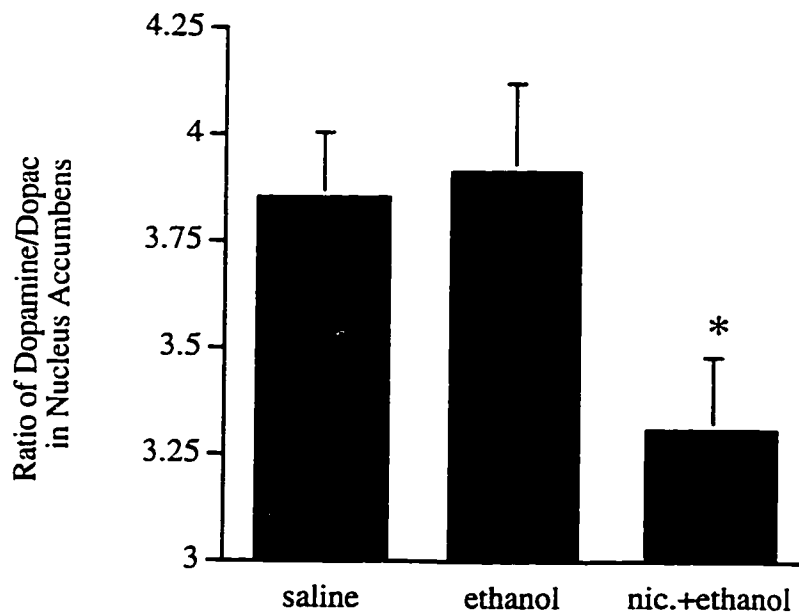
Figure 9b. Ratio of Dopamine/DOPAC in nucleus accumbens following treatment with saline, ethanol. or nicotine+ethanol. Subjects received nicotine during conditioning. (Means and S.E.M.)

* Differs from ethanol group (p<.05)

Figure 10a. Levels of Dopamine and DOPAC in nucleus accumbens following treatment with saline, ethanol. or nicotine+ethanol. Subjects received saline during conditioning. (Means and S.E.M.)
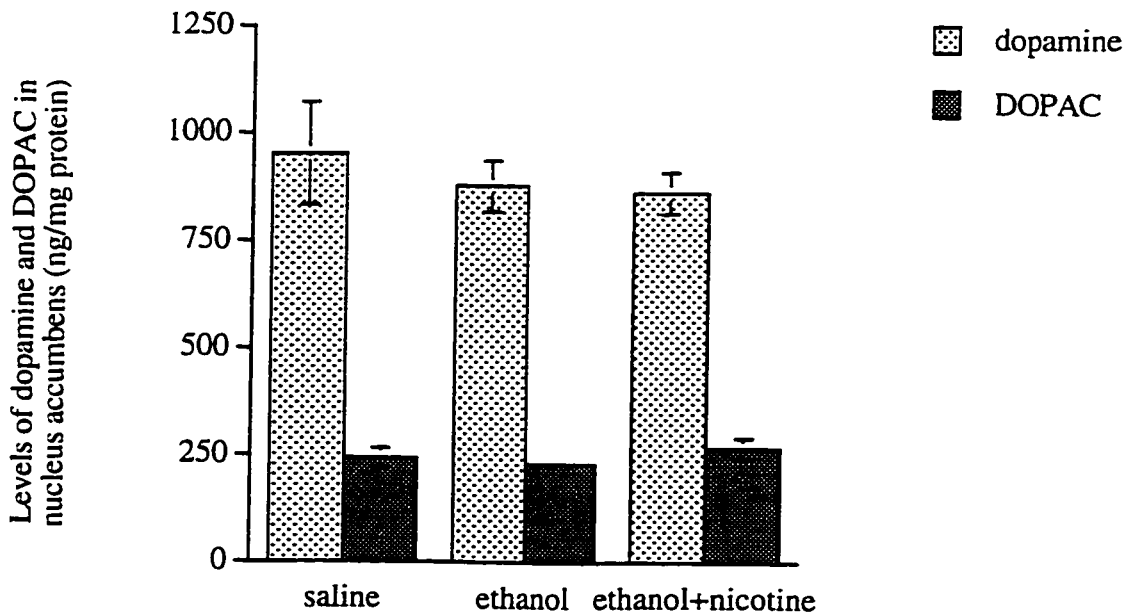

Figure 10b. Levels of Dopamine and DOPAC in nucleus accumbens following treatment with saline, ethanol. or nicotine+ethanol. Subjects received nicotine during conditioning. (Means and S.E.M.)
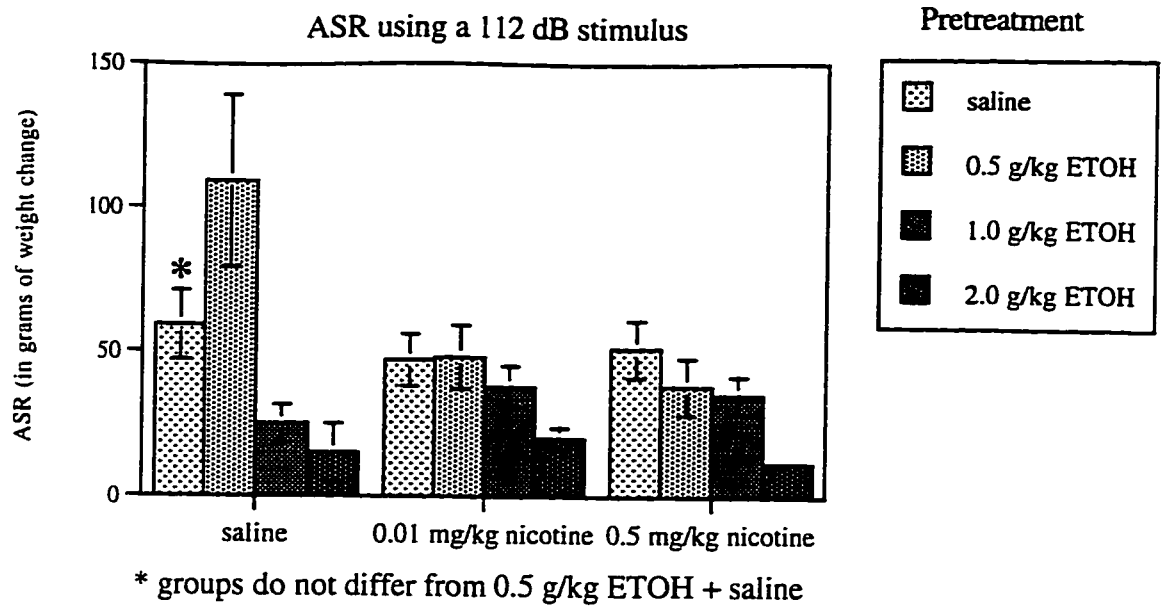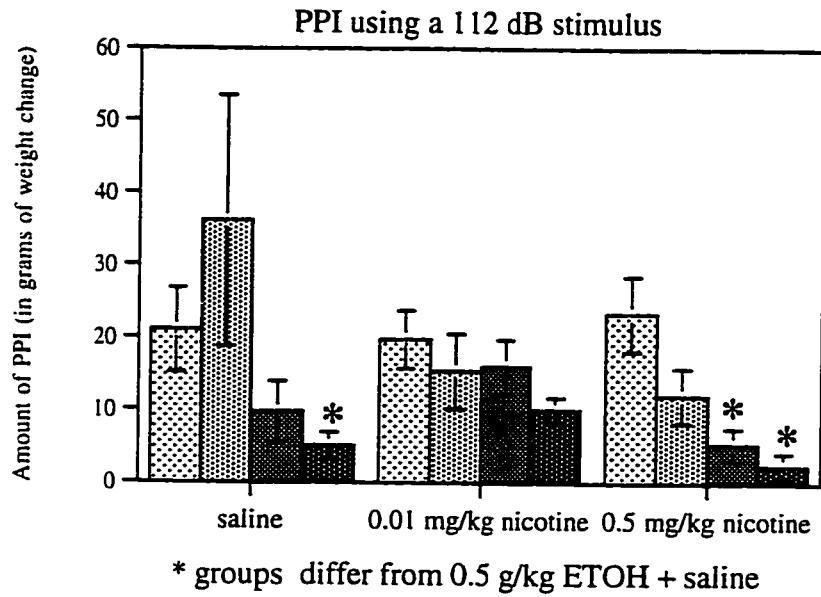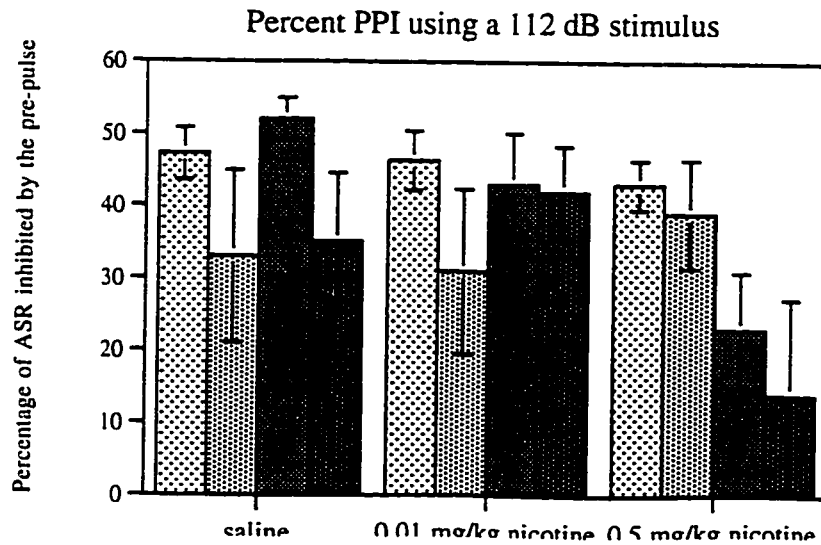
ASR using a 112 dB stimulus

Figure 11a

* groups do not differ from 0.5 g/kg ETOH + saline

Pretreatment

saline
0.5 g/kg ETOH
1.0 g/kg ETOH
2.0 g/kg ETOH

PPI using a 112 dB stimulus

Figure 11b

* groups differ from 0.5 g/kg ETOH + saline

Percent PPI using a 112 dB stimulus

Figure 11c

ASR using a 122 dB stimulus

Figure 12a

* groups differ from saline/saline

† groups differ from 0.5 g/kg ETOH/saline

Pretreatment
- saline
- 0.5 g/kg ETOH
- 1.0 g/kg ETOH
- 2.0 g/kg ETOH

PPI using a 122 dB stimulus

Figure 12b

Percent PPI using a 122 dB stimulus

Figure 12c

ng/mg protein

Figure 13a

Nicotine Dose

Dopamine

ng/mg protein

Figure 13c

Nicotine Dose

DOPAC

ng/mg protein

Figure 13b

Nicotine Dose

L-DOPA

ng/mgprotein

Figure 13d

Nicotine Dose

HVA

Ethanol Dose

saline

0.5 g/kg ETOH

1.0 g/kg ETOH
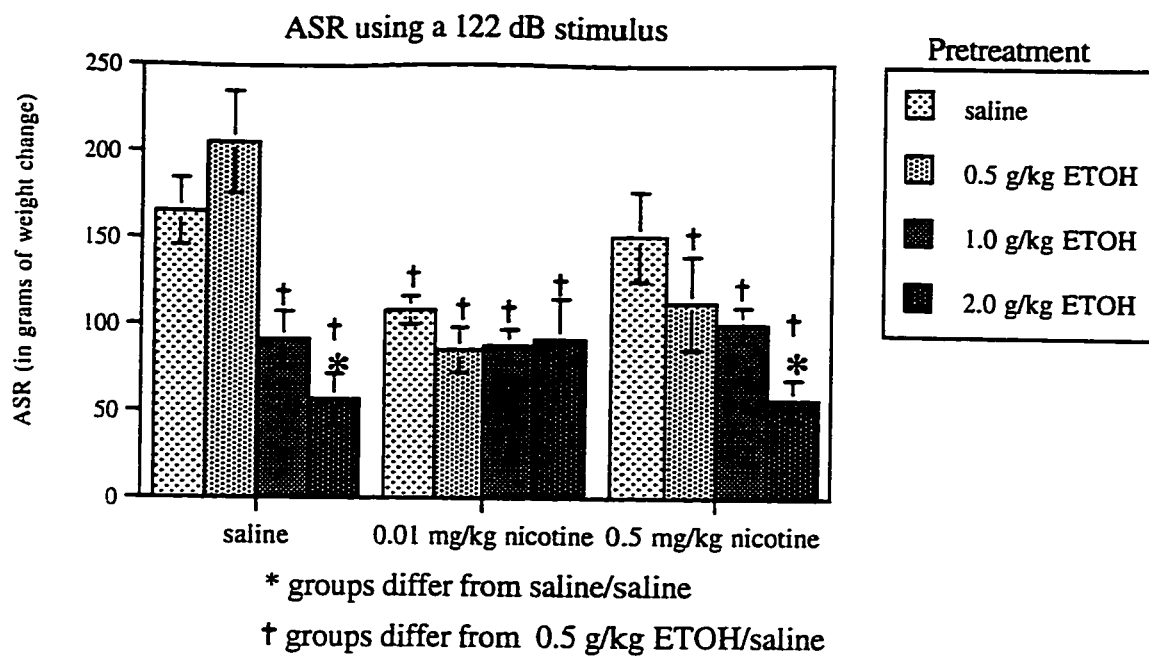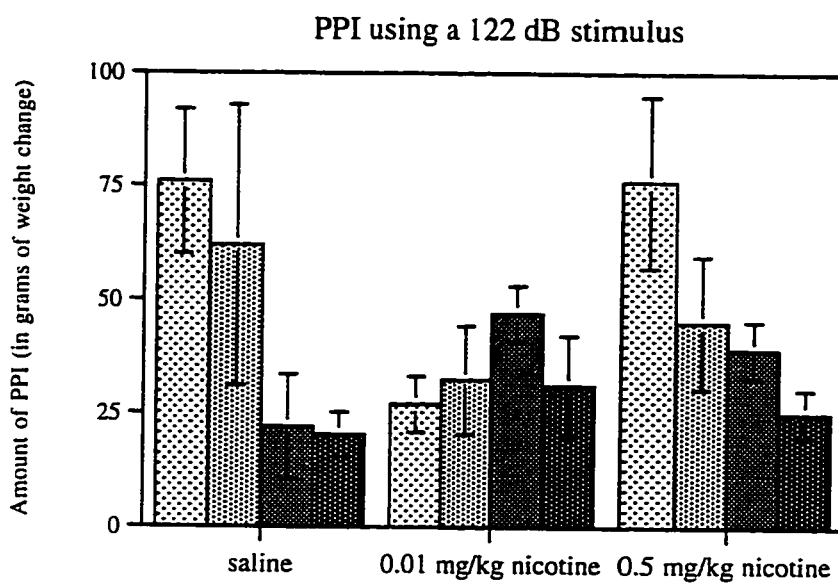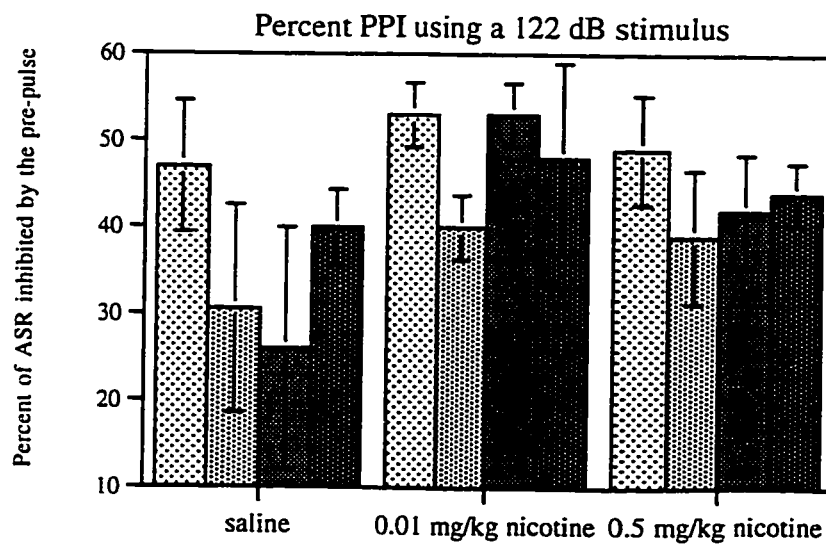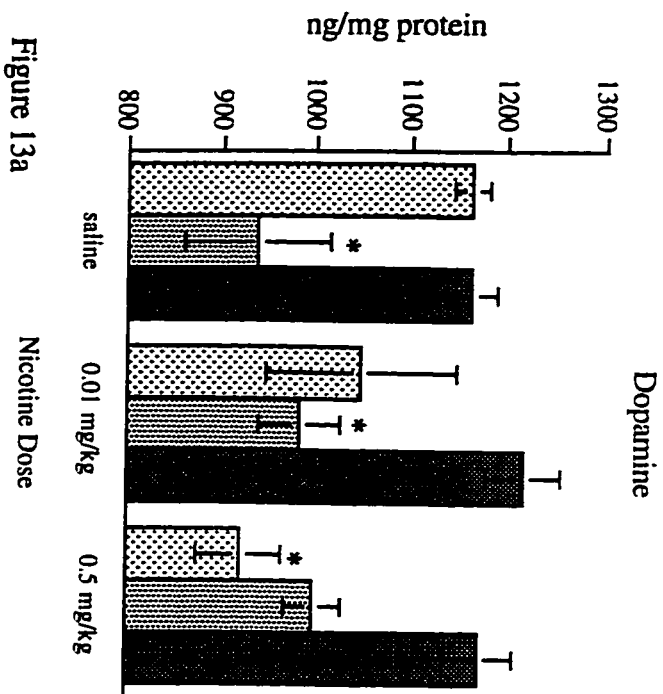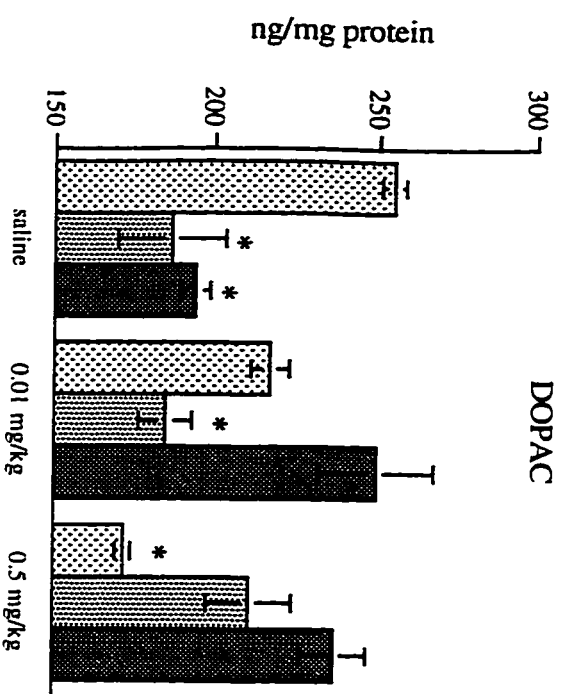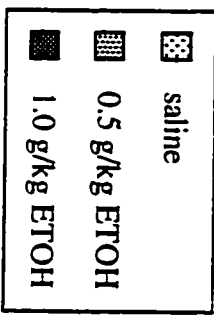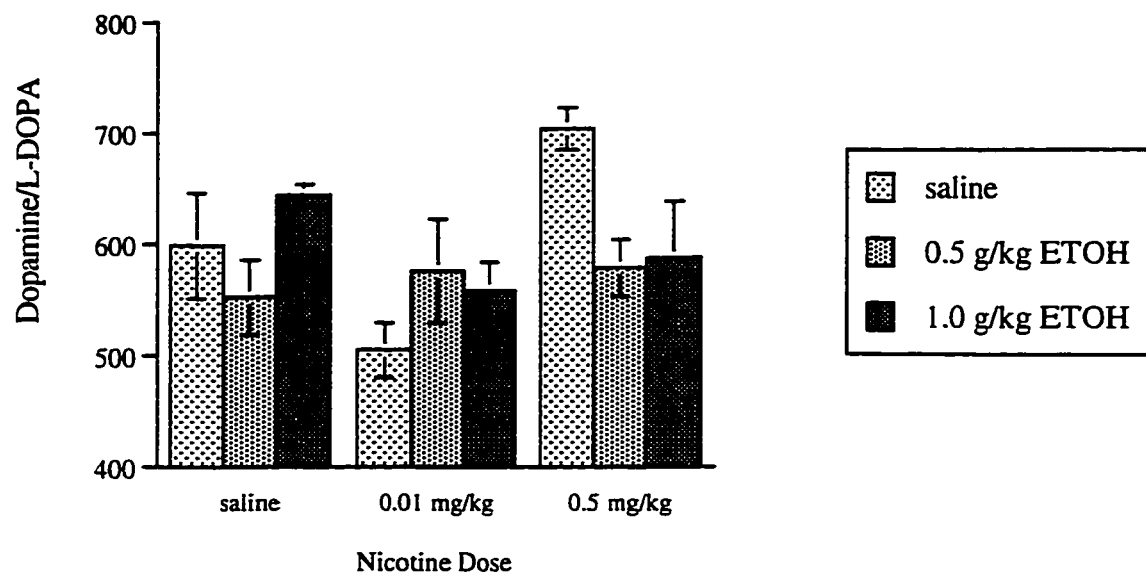
Figure 14a

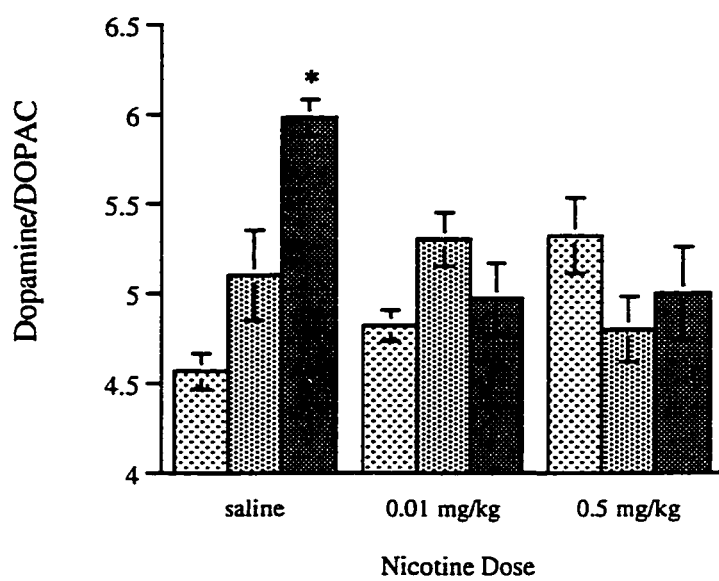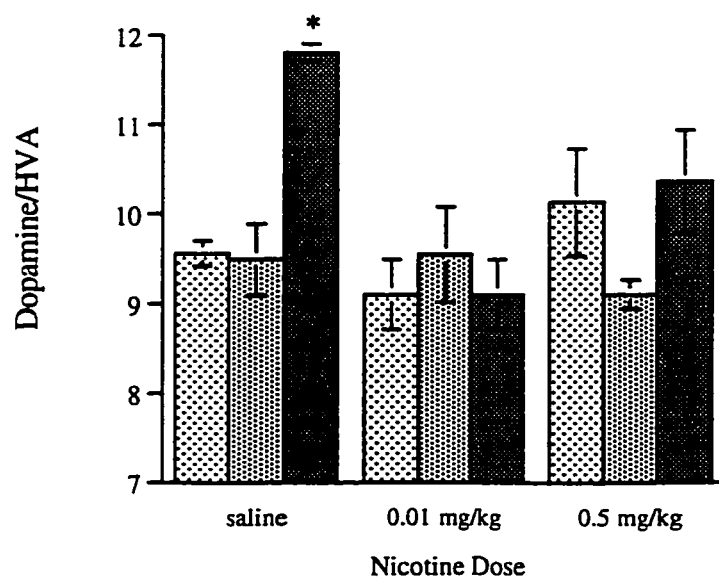
Figure 14b

Figure 14c

APPENDICES

Appendix A. HPLC assay procedure for the measurement of l-DOPA,

dopamine, DOPAC, and HVA in nucleus accumbens (Experiment

1) or striatum (Experiment 2) based on the procedures of Shoami,

et al., (1983) and Zuddas, et al., (1989)

Preparation of Tissue.

Rats were decapitated without anesthesia and the nucleus accumbens

(Experiment 1) or the striatum (Experiment 2) were removed. These tissues

were weighed and then homogenized in 500-600 µl extraction solution

containing 0.1M perchloric acid, 1.0% Ethanol, and 0.02%

ethylenediaminetetraacetic acid (EDTA) in a ratio of 110:100:100. 100 µl

aliquots of tissue homogenate were removed for later protein analysis (see

Appendix B) and the remaining tissue homogenate was cold centrifuged at

15,000 RPM for 15 minutes. After being centrifuged, supernatant was

transferred to 0.5 ml filtration tubes (0.45 µm HVPP; Millipore Corporation,

Bedford, MA) and centrifuged for an additional 30 seconds using a bench-top

microcentrifuge. 100 µl aliquots of the resulting, filtered supernatant were

transferred to 0.25 ml microcentrifuge tubes (PGC Scientific, Gaithersburg, MD)

and frozen for later injection into the HPLC column.

Measurement of DOPA, dopamine, DOPAC, and HVA was performed

using a high-performance liquid chromatograph (HPLC) consisting of a 4.6 mm

x 25 mm reverse phase column (C-18 Beckman ultrasphere), maintained at

36°C, a WISP 717 plus automatic sampler (Waters Associates, Milford, MA),

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<u>Appendix A</u>. (cont.)

and an amperometric electrochemical detector (Antec Leyden Inc., The

Netherlands). The mobile phase contained, in 2.0 L of distilled water, 2.2 g of

heptane sulfonic acid, 0.17 g of EDTA, 15 ml of triethylamine, 9 ml of 85%

phosphoric acid (pH=2.55) and 50 ml of acetonitrile. The mobile phase was

filtered anddegassed and was delivered at a rate of 0.8 ml/min. The applied

potential was set to 0.780 V. Results were recorded using a millennium

chromatography manager (Waters Associates, Milford, MA) and quantified

using an external standard. Values are expressed as ng/mg protein. Protein

content of each sample was analyzed using a BCA micro assay kit as described

in Appendix B.

Appendix B. Assay for measurement of proteins in brain based on Pierce Micro BCA Protein Assay Reagent Kit; Cat. #23235

Tissue was homogenized in 500-600 µl of extraction solution as described in Appendix A. 50 µl aliquots of tissue homogenate were cold centrifuged at 15,000 RPM for 20 minutes. The supernatant was carefully removed and the remaining pellet was resuspended in 300 µl of deionized H_2O+NaOH (pH adjusted to 11.3). 10 µl of resuspended sample was then transferred, in duplicate, to 96 well plates. 300 µl of "working reagent" was added to each sample and to each of seven standards (standards were prepared fresh from stock bovine serum albumen (2 mg/ml) in concentrations that ranged from 0-1200 µg/ml). Working reagent was prepared fresh as directed in Pierce Micro BCA Assay Reagent Kit; Cat. # 23235). Each 96-well plate was shaken gently for 30 seconds using a bench-top shaker/vortexer before incubating. Incubations were performed inside a CERES 900 automatic plate reader (Bio-Tek Instruments, Inc., Winooski, VT) at 37° C for 30 minutes. Following the incubation the plate covers were removed and the samples were read using 570 nm optical filter.

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IMAGE EVALUATION
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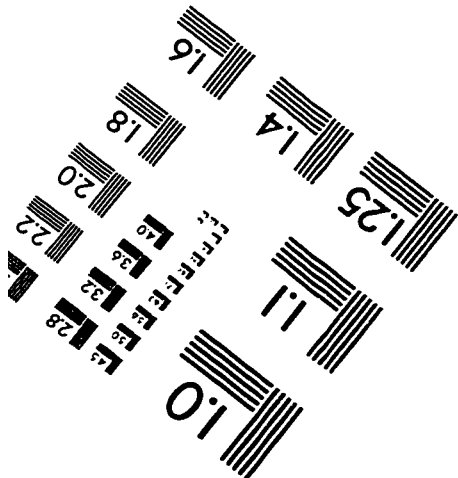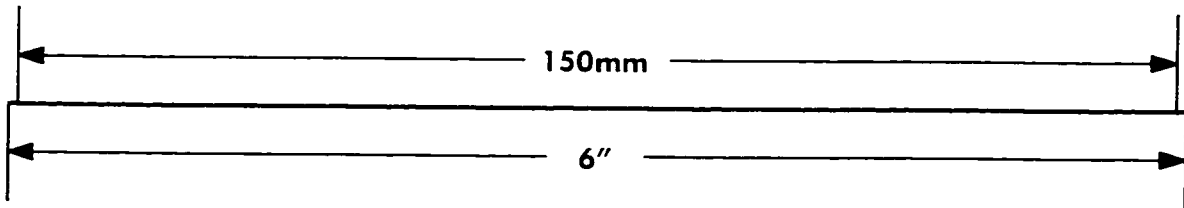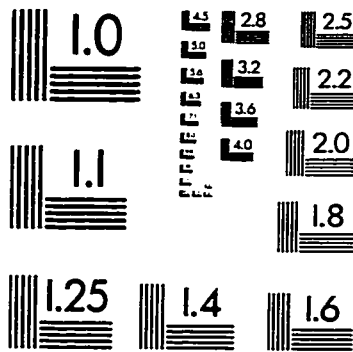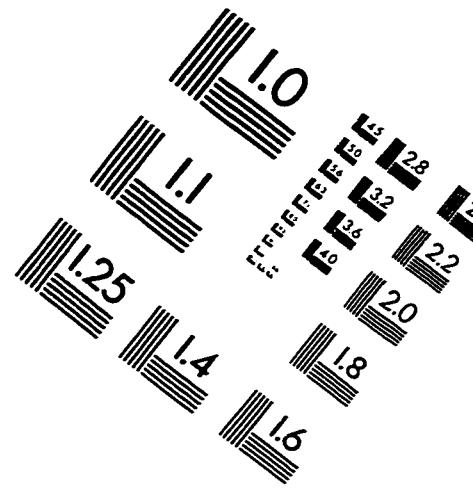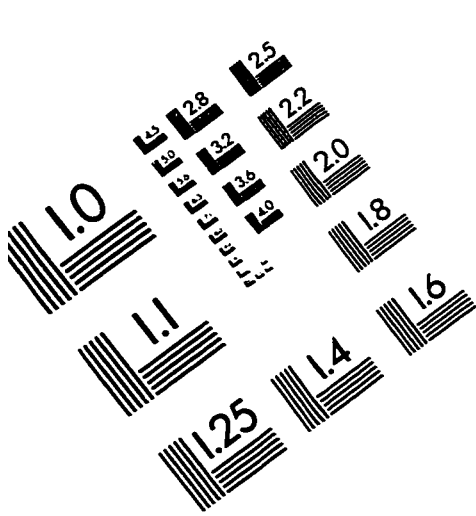
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